EPA/600/R-16b/305b
Agency Review Draft
www.epa.gov/iris

Toxicological Review of Hexabromocyclododecane

[CASRN 3194-55-6]

Supplemental Information

December 2017

NOTICE

This document is an **Agency Review Draft.** This information is distributed solely for the purpose of pre-dissemination peer review under applicable information quality guidelines. It has not been formally disseminated by EPA. It does not represent and should not be construed to represent any Agency determination or policy. It is being circulated for review of its technical accuracy and science policy implications.

Integrated Risk Information System
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

This document is a preliminary draft for review purposes only. This information is distributed solely for the purpose of pre-dissemination peer review under applicable information quality guidelines. It has not been formally disseminated by EPA. It does not represent and should not be construed to represent any Agency determination or policy. Mention of trade names or commercial products does not constitute endorsement of recommendation for use.

ED_005297A_00166423-00002

CONTENTS

ED_005297A_00166423-00003

TABLES

ED_005297A_00166423-00004

FIGURES

[TOC \h \z \t "_IR Figure Caption" \c]

ED_005297A_00166423-00005

ABBREVIATIONS

AGD	anogenital distance
AIC	Akaike's information criterion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
atm	atmosphere
BAEP	brainstem evoked auditory potential
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMI	body mass index
BMR	benchmark response
BW	body weight
CAR	constitutive androstane receptor
CASRN	Chemical Abstracts Service Registry Number
CGN	cerebellar granule neuron
CHO	Chinese hamster ovary (cell line cells)
CI	confidence interval
df	degrees of freedom
DAF	dosimetric adjustment factor
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
ER	extra risk
FOB	functional observational battery
FSH	follicle-stimulating hormone
GD	gestation day
GGT	γ-glutamyl transferase
GI	gastrointestinal
GLP	good laboratory practices
HBCD	hexabromocyclododecane
HED	human equivalent dose
HERO	Health and Environmental Research Online
HOME	Home Observation for Measurement of the Environment
HPT	hypothalamic-pituitary-thyroid
IgG	immunoglobulin G
IgM	immunoglobulin M
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
i.v.	intravenous
KLH	keyhole limpet hemocyanin
LC	liquid chromatography
LGCC	ligand-gated Ca2+ channel
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOQ	limit of quantitation
MOA	mode of action
mRNA	messenger ribonucleic acid
MS	mass spectrometry
NCEA	National Center for Environmental Assessment
NK	natural killer
NOAEL	no-observed-adverse-effect level
OECD	Organisation for Economic Co-operation and Development
ORD	Office of Research and Development
PBDE	polybrominated diphenyl ether
PBPK	physiologically based pharmacokinetic
PCB	polychlorinated biphenyl
PND	postnatal day
PNM	postnatal month
PNW	postnatal week
POD	point of departure
POD_{ADJ}	duration-adjusted POD
PPAR	peroxisome proliferator-activated receptor
PXR	pregnane X receptor
RD	relative deviation
RfC	inhalation reference concentration
RfD	oral reference dose
ROS	reactive oxygen species
SD	standard deviation
SE	standard error
SERCA	sarco-endoplasmic reticulum Ca2+-dependent ATPase
SHBG	sex hormone binding globulin
SRBC	sheep red blood cell
T3	triiodothyronine
T4	thyroxine
TR	thyroid response
TRE	thyroid hormone response element
TSCA	Toxic Substances Control Act
TSCATS	Toxic Substances Control Act Test Submissions
TSH	thyroid-stimulating hormone
TTR	transthyretin
TWA	time-weighted average
UF	uncertainty factor
UFA	animal-to-human uncertainty factor
UFD	database deficiencies uncertainty factor
UFH	human variation uncertainty factor
UFL	LOAEL-to-NOAEL uncertainty factor
UFS	subchronic-to-chronic uncertainty factor
UGT	uridine diphosphate glucuronyl transferase
VGCC	voltage-gated Ca2+ channel
WBC	white blood cell
WOS	Web of Science

ED_005297A_00166423-00006

APPENDIX A. ASSESSMENTS BY OTHER NATIONAL AND INTERNATIONAL HEALTH AGENCIES

Table A-1. Assessments by other national and international health agencies

Organization	Toxicity value
{NICNAS, 2012, 1443965@@author-year} *Hexabromocyclododecane: Priority existing chemical assessment report no. 34.*	NOAEL compared to estimated daily intakes to determine a margin of exposure NOAEL = 10.2 mg/kg-d, based on reproductive effects in a two-generation reproductive toxicity rat study {Ema, 2008, 787657}.
{EFSA, 2011, 3445685@@author-year} *Scientific Opinion on Hexabromocyclododecanes (HBCDDs) in Food.*	$BMDL_{10}$ compared to estimated daily intakes $BMDL_{10}$ = 0.93 mg/kg for neurobehavioral effects in mice observed 90 d after a single dose on PND 10 {Eriksson, 2006, 787660}; the $BMDL_{10}$ was adjusted by an absorption fraction of 0.085 to obtain an adjusted body burden of 0.79 mg/kg BW.
{Environment Canada, 2011, 1937209@@author-year} *Screening assessment report on hexabromocyclododecane. Chemical Abstracts Service Registry Number 3194-55-6.*	NOAELs compared to estimated daily intakes NOAEL = 10 mg/kg-day, based on two-generation reproductive toxicity study {Ema, 2008, 787657}. Infants and children: LOAEL = 0.9 mg/kg, based on neurobehavioral effects in mice observed 90 days after treatment with a single dose of HBCD on PND 10 {Eriksson, 2006, 787660}.
{EINECS, 2008, 1443914@@author-year} *Risk assessment: Hexabromocyclododecane. CAS-No.: 25637-99-4.*	NOAELs compared to estimated daily intakes Repeat-dose toxicity: NOAEL = 22.9 mg/kg-day, based on liver weight increase in rats orally exposed for 28 days {van der Ven, 2006, 787745}. Reproductive toxicity/fertility: NOAEL = 10 mg/kg-day, based on decreased fertility index and reduced number of primordial follicles in a two-generation rat study {Ema, 2008, 787657}. Carcinogenicity assessment: "Based on the only available lifetime bioassay, it is not possible to assess the carcinogenic potential of HBCDD. However, the available data (including mutagenicity) gives no reason for further exploration of this endpoint."

BMDL = benchmark dose lower confidence limit; BW= body weight; CAS = Chemical Abstracts Service; HBCDD = hexabromocyclododecane; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; PND = postnatal day

APPENDIX B. ADDITIONAL DETAILS OF SYSTEMATIC REVIEW METHODS

Commented [RS1]: This appendix will be revised; we anticipate that details of the systematic review methods will be moved into the stand-along assessment protocol.

B.1 LITERATURE SEARCH AND SCREENING STRATEGY

The literature search for hexabromocyclododecane (HBCD) was conducted in four online scientific databases through July 2016. The detailed search strategy used to search these databases is provided in Table B-1. The computerized database searches were augmented by review of online regulatory sources, as well as "forward" and "backward" Web of Science (WOS) searches of four primary toxicology studies (Table B-2). Forward searching was used to identify articles that cited the four selected studies in Table B-2 and backward searching was used to identify articles that the selected studies cited.

Commented [SR2]: NOTE: We plan to incorporate the literature search OPPT performed from January 1, 2016 to February 14, 2017.

Table B-1. Literature search query strings for computerized databases

Database search date	Terms	Hits
PubMed 07/12/16	(3194-55-6[rn] OR 25637-99-4[rn] OR "1,2,5,6,9,10-hexabromocyclodecane"[tw] OR hexabromocyclododecane*[tw] OR hbcd*[tw] OR "Bromkal 73-6CD"[tw] OR "Bromkal 73-6D"[tw] OR "HBCD-LM"[tw] OR "HBCD-LMS"[tw] OR "HBCD-SP 75"[tw] OR "Myflam 11645"[tw] OR "Nicca Fi-None CG 1"[tw] OR "Nicca Fi-None TS 1"[tw] OR "Nicca Fi-None TS 3"[tw] OR "Nicca Fi-None TS 88"[tw] OR "Pyroguard F 800"[tw] OR "Pyroguard SR 103"[tw] OR "Pyroguard SR 103A"[tw] OR "Pyroguard SR 103HR"[tw] OR "Pyroguard SR 104"[tw] OR "Pyrovatex 3887"[tw] OR "Safron 5261"[tw] OR "Saytex HBCD"[tw] OR "Saytex HBCD-LM"[tw] OR "Saytex HBCD-SF"[tw] OR "Saytex HP 900"[tw] OR "Saytex HP 900G"[tw]) AND (2014/11/01:3000[mhda] OR 2014/11/01:3000[edat] OR 2014/11/01:3000[crdat])	186
11/14/14	(3194-55-6[rn] OR 25637-99-4[rn] OR "1,2,5,6,9,10-hexabromocyclodecane"[tw] OR hexabromocyclododecane*[tw] OR hbcd*[tw] OR "Bromkal 73-6CD"[tw] OR "Bromkal 73-6D"[tw] OR "HBCD-LM"[tw] OR "HBCD-LMS"[tw] OR "HBCD-SP 75"[tw] OR "Myflam 11645"[tw] OR "Nicca Fi-None CG 1"[tw] OR "Nicca Fi-None TS 1"[tw] OR "Nicca Fi-None TS 3"[tw] OR "Nicca Fi-None TS 88"[tw] OR "Pyroguard F 800"[tw] OR "Pyroguard SR 103"[tw] OR "Pyroguard SR 103A"[tw] OR "Pyroguard SR 103HR"[tw] OR "Pyroguard SR 104"[tw] OR "Pyrovatex 3887"[tw] OR "Safron 5261"[tw] OR "Saytex HBCD"[tw] OR "Saytex HBCD-LM"[tw] OR "Saytex HBCD-SF"[tw] OR "Saytex HP 900"[tw] OR "Saytex HP 900G"[tw]) AND (2014/05/01:3000[mhda] OR 2014/05/01:3000[edat] OR 2014/05/01:3000[crdat])	77
06/09/14	(3194-55-6[rn] OR 25637-99-4[rn] OR "1,2,5,6,9,10-hexabromocyclodecane"[tw] OR hexabromocyclododecane*[tw] OR hbcd*[tw] OR "Bromkal 73-6CD"[tw] OR "Bromkal 73-6D"[tw] OR "HBCD-LM"[tw] OR "HBCD-LMS"[tw] OR "HBCD-SP 75"[tw] OR "Myflam 11645"[tw] OR "Nicca Fi-None CG 1"[tw] OR "Nicca Fi-None TS 1"[tw] OR "Nicca Fi-None TS 3"[tw] OR "Nicca Fi-None TS 88"[tw] OR "Pyroguard F 800"[tw] OR "Pyroguard SR 103"[tw] OR "Pyroguard SR 103A"[tw] OR "Pyroguard SR 103HR"[tw] OR "Pyroguard SR 104"[tw] OR	115

ED_005297A_00166423-00008

Database search date	Terms	Hits
	"Pyrovatex 3887"[tw] OR "Safron 5261"[tw] OR "Saytex HBCD"[tw] OR "Saytex HBCD-LM"[tw] OR "Saytex HBCD-SF"[tw] OR "Saytex HP 900"[tw] OR "Saytex HP 900G"[tw]) AND (2013/06/01:3000[mhda] OR 2013/06/01:3000[edat] OR 2013/06/01:3000[crdat])	
08/20/13	hexabromocyclododecane[nm] OR "3194-55-6"[tw] OR "25637-99-4"[tw] OR "1,2,5,6,9,10-hexabromocyclodecane"[tw] OR hexabromocyclododecane*[tw] OR hbcd[tw] OR hbcds[tw]	468
Web of Science 07/12/16	(TS="Bromkal 73-6CD" OR TS="Bromkal 73-6D" OR TS="HBCD-LM" OR TS="HBCD-LMS" OR TS="HBCD-SP 75" OR TS="Myflam 11645" OR TS="Nicca Fi-None CG 1" OR TS="Nicca Fi-None TS 1" OR TS="Nicca Fi-None TS 3" OR TS="Nicca Fi-None TS 88" OR TS="Pyroguard F 800" OR TS="Pyroguard SR 103" OR TS="Pyroguard SR 103A" OR TS="Pyroguard SR 103HR" OR TS="Pyroguard SR 104" OR TS="Pyrovatex 3887" OR TS="Safron 5261" OR TS="Saytex HBCD" OR TS="Saytex HBCD-LM" OR TS="Saytex HBCD-SF" OR TS="Saytex HP 900" OR TS="Saytex HP 900G" OR TS="1,2,5,6,9,10-hexabromocyclodecane" OR TS=hexabromocyclododecane* OR TS=hbcd*) AND ((WC=("Toxicology" OR "Endocrinology & Metabolism" OR "Gastroenterology & Hepatology" OR "Gastroenterology & Hepatology" OR "Hematology" OR "Neurosciences" OR "Obstetrics & Gynecology" OR "Pharmacology & Pharmacy" OR "Physiology" OR "Respiratory System" OR "Urology & Nephrology" OR "Anatomy & Morphology" OR "Andrology" OR "Pathology" OR "Otorhinolaryngology" OR "Ophthalmology" OR "Pediatrics" OR "Oncology" OR "Reproductive Biology" OR "Developmental Biology" OR "Biology" OR "Dermatology" OR "Allergy" OR "Public, Environmental & Occupational Health") OR SU=("Anatomy & Morphology" OR "Cardiovascular System & Cardiology" OR "Developmental Biology" OR "Endocrinology & Metabolism" OR "Gastroenterology & Hepatology" OR "Hematology" OR "Immunology" OR "Neurosciences & Neurology" OR "Obstetrics & Gynecology" OR "Oncology" OR "Ophthalmology" OR "Pathology" OR "Pediatrics" OR "Pharmacology & Pharmacy" OR "Physiology" OR "Public, Environmental & Occupational Health" OR "Respiratory System" OR "Toxicology" OR "Urology & Nephrology" OR "Reproductive Biology" OR "Dermatology" OR "Allergy")) OR (WC="veterinary sciences" AND (TS="rat" OR TS="rats" OR TS="mouse" OR TS="murine" OR TS="mice" OR TS="guinea" OR TS="muridae" OR TS=rabbit* OR TS=lagomorph* OR TS=hamster* OR TS=ferret* OR TS=gerbil* OR TS=rodent* OR TS="dog" OR TS="dogs" OR TS=beagle* OR TS="canine" OR TS="cats" OR TS="feline" OR TS="pig" OR TS="pigs" OR TS="swine" OR TS="porcine" OR TS=monkey* OR TS=macaque* OR TS=baboon* OR TS=marmoset*)) OR (TS=toxic* AND (TS="rat" OR TS="rats" OR TS="mouse" OR TS="murine" OR TS="mice" OR TS="guinea" OR TS="muridae" OR TS=rabbit* OR TS=lagomorph* OR TS=hamster* OR TS=ferret* OR TS=gerbil* OR TS=rodent* OR TS="dog" OR TS="dogs" OR TS=beagle* OR TS="canine" OR TS="cats" OR TS="feline" OR TS="pig" OR TS="pigs" OR TS="swine" OR TS="porcine" OR TS=monkey* OR TS=macaque* OR TS=baboon* OR TS=marmoset* OR TS="child" OR TS="children" OR TS=adolescen* OR TS=infant* OR TS="WORKER" OR TS="WORKERS" OR TS="HUMAN" OR TS=patient* OR TS=mother OR TS=fetal OR TS=fetus OR TS=citizens OR TS=milk OR TS=formula)) OR TI=toxic*) Limit 2014-present	100
11/14/14	(TS="Bromkal 73-6CD" OR TS="Bromkal 73-6D" OR TS="HBCD-LM" OR TS="HBCD-LMS" OR TS="HBCD-SP 75" OR TS="Myflam 11645" OR TS="Nicca Fi-None CG 1" OR TS="Nicca Fi-None TS 1" OR TS="Nicca Fi-None TS 3" OR TS="Nicca Fi-None TS 88" OR TS="Pyroguard F 800" OR TS="Pyroguard SR 103" OR TS="Pyroguard SR 103A" OR TS="Pyroguard SR 103HR" OR TS="Pyroguard SR 104" OR TS="Pyrovatex 3887" OR TS="Safron 5261" OR TS="Saytex HBCD" OR TS="Saytex HBCD-LM" OR TS="Saytex HBCD-SF" OR TS="Saytex HP 900" OR TS="Saytex HP 900G" OR TS="1,2,5,6,9,10-hexabromocyclodecane" OR	80

ED_005297A_00166423-00009

Database search date	Terms	Hits
	TS=hexabromocyclododecane* OR TS=hbcd*) AND ((WC=("Toxicology" OR "Endocrinology & Metabolism" OR "Gastroenterology & Hepatology" OR "Gastroenterology & Hepatology" OR "Hematology" OR "Neurosciences" OR "Obstetrics & Gynecology" OR "Pharmacology & Pharmacy" OR "Physiology" OR "Respiratory System" OR "Urology & Nephrology" OR "Anatomy & Morphology" OR "Andrology" OR "Pathology" OR "Otorhinolaryngology" OR "Ophthalmology" OR "Pediatrics" OR "Oncology" OR "Reproductive Biology" OR "Developmental Biology" OR "Biology" OR "Dermatology" OR "Allergy" OR "Public, Environmental & Occupational Health") OR SU=("Anatomy & Morphology" OR "Cardiovascular System & Cardiology" OR "Developmental Biology" OR "Endocrinology & Metabolism" OR "Gastroenterology & Hepatology" OR "Hematology" OR "Immunology" OR "Neurosciences & Neurology" OR "Obstetrics & Gynecology" OR "Oncology" OR "Ophthalmology" OR "Pathology" OR "Pediatrics" OR "Pharmacology & Pharmacy" OR "Physiology" OR "Public, Environmental & Occupational Health" OR "Respiratory System" OR "Toxicology" OR "Urology & Nephrology" OR "Reproductive Biology" OR "Dermatology" OR "Allergy")) OR (WC="veterinary sciences" AND (TS="rat" OR TS="rats" OR TS="mouse" OR TS="murine" OR TS="mice" OR TS="guinea" OR TS="muridae" OR TS=rabbit* OR TS=lagomorph* OR TS=hamster* OR TS=ferret* OR TS=gerbil* OR TS=rodent* OR TS="dog" OR TS="dogs" OR TS=beagle* OR TS="canine" OR TS="cats" OR TS="feline" OR TS="pig" OR TS="pigs" OR TS="swine" OR TS="porcine" OR TS=monkey* OR TS=macaque* OR TS=baboon* OR TS=marmoset*)) OR (TS=toxic* AND (TS="rat" OR TS="rats" OR TS="mouse" OR TS="murine" OR TS="mice" OR TS="guinea" OR TS="muridae" OR TS=rabbit* OR TS=lagomorph* OR TS=hamster* OR TS=ferret* OR TS=gerbil* OR TS=rodent* OR TS="dog" OR TS="dogs" OR TS=beagle* OR TS="canine" OR TS="cats" OR TS="feline" OR TS="pig" OR TS="pigs" OR TS="swine" OR TS="porcine" OR TS=monkey* OR TS=macaque* OR TS=baboon* OR TS=marmoset*) OR (TS="child" OR TS="children" OR TS=adolescen* OR TS=infant* OR TS="WORKER" OR TS="WORKERS" OR TS="HUMAN" OR TS=patient* OR TS=mother OR TS=fetal OR TS=fetus OR TS=citizens OR TS=milk OR TS=formula)) OR TI=toxic*) Limit 2013-present	
06/09/14	(TS="Bromkal 73-6CD" OR TS="Bromkal 73-6D" OR TS="HBCD-LM" OR TS="HBCD-LMS" OR TS="HBCD-SP 75" OR TS="Myflam 11645" OR TS="Nicca Fi-None CG 1" OR TS="Nicca Fi-None TS 1" OR TS="Nicca Fi-None TS 3" OR TS="Nicca Fi-None TS 88" OR TS="Pyroguard F 800" OR TS="Pyroguard SR 103" OR TS="Pyroguard SR 103A" OR TS="Pyroguard SR 103HR" OR TS="Pyroguard SR 104" OR TS="Pyrovatex 3887" OR TS="Safron 5261" OR TS="Saytex HBCD" OR TS="Saytex HBCD-LM" OR TS="Saytex HBCD-SF" OR TS="Saytex HP 900" OR TS="Saytex HP 900G" OR TS="1,2,5,6,9,10-hexabromocyclodecane" OR TS=hexabromocyclododecane* OR TS=hbcd*) AND ((WC=("Toxicology" OR "Endocrinology & Metabolism" OR "Gastroenterology & Hepatology" OR "Gastroenterology & Hepatology" OR "Hematology" OR "Neurosciences" OR "Obstetrics & Gynecology" OR "Pharmacology & Pharmacy" OR "Physiology" OR "Respiratory System" OR "Urology & Nephrology" OR "Anatomy & Morphology" OR "Andrology" OR "Pathology" OR "Otorhinolaryngology" OR "Ophthalmology" OR "Pediatrics" OR "Oncology" OR "Reproductive Biology" OR "Developmental Biology" OR "Biology" OR "Dermatology" OR "Allergy" OR "Public, Environmental & Occupational Health") OR SU=("Anatomy & Morphology" OR "Cardiovascular System & Cardiology" OR "Developmental Biology" OR "Endocrinology & Metabolism" OR "Gastroenterology & Hepatology" OR "Hematology" OR "Immunology" OR "Neurosciences & Neurology" OR "Obstetrics & Gynecology" OR "Oncology" OR "Ophthalmology" OR "Pathology" OR "Pediatrics" OR "Pharmacology & Pharmacy" OR "Physiology" OR "Public, Environmental & Occupational Health" OR "Respiratory System" OR "Toxicology" OR "Urology & Nephrology" OR "Reproductive Biology" OR "Dermatology"	57

Database search date	Terms	Hits
	OR "Allergy")) OR (WC="veterinary sciences" AND (TS="rat" OR TS="rats" OR TS="mouse" OR TS="murine" OR TS="mice" OR TS="guinea" OR TS="muridae" OR TS=rabbit* OR TS=lagomorph* OR TS=hamster* OR TS=ferret* OR TS=gerbil* OR TS=rodent* OR TS="dog" OR TS="dogs" OR TS=beagle* OR TS="canine" OR TS="cats" OR TS="feline" OR TS="pig" OR TS="pigs" OR TS="swine" OR TS="porcine" OR TS=monkey* OR TS=macaque* OR TS=baboon* OR TS=marmoset*)) OR (TS=toxic* AND (TS="rat" OR TS="rats" OR TS="mouse" OR TS="murine" OR TS="mice" OR TS="guinea" OR TS="muridae" OR TS=rabbit* OR TS=lagomorph* OR TS=hamster* OR TS=ferret* OR TS=gerbil* OR TS=rodent* OR TS="dog" OR TS="dogs" OR TS=beagle* OR TS="canine" OR TS="cats" OR TS="feline" OR TS="pig" OR TS="pigs" OR TS="swine" OR TS="porcine" OR TS=monkey* OR TS=macaque* OR TS=baboon* OR TS=marmoset*) OR (TS="child" OR TS="children" OR TS=adolescen* OR TS=infant* OR TS="WORKER" OR TS="HUMAN" OR TS=patient* OR TS=mother OR TS=fetal OR TS=citizens OR TS=milk OR TS=formula OR TS=diet)) OR TI=toxic*) Limit 2013 to present	
08/21/13	(TS="1,2,5,6,9,10-hexabromocyclodecane" OR TS="hexabromocyclododecane" OR TS=hexabromocyclododecane* OR TS="HBCD" OR TS="HBCDs") AND ((WC=("Toxicology" OR "Endocrinology & Metabolism" OR "Gastroenterology & Hepatology" OR "Gastroenterology & Hepatology" OR "Hematology" OR "Neurosciences" OR "Obstetrics & Gynecology" OR "Pharmacology & Pharmacy" OR "Physiology" OR "Respiratory System" OR "Urology & Nephrology" OR "Anatomy & Morphology" OR "Andrology" OR "Pathology" OR "Otorhinolaryngology" OR "Ophthalmology" OR "Pediatrics" OR "Oncology" OR "Reproductive Biology" OR "Developmental Biology" OR "Biology" OR "Dermatology" OR "Allergy" OR "Public, Environmental & Occupational Health") OR SU=("Anatomy & Morphology" OR "Cardiovascular System & Cardiology" OR "Developmental Biology" OR "Endocrinology & Metabolism" OR "Gastroenterology & Hepatology" OR "Hematology" OR "Immunology" OR "Neurosciences & Neurology" OR "Obstetrics & Gynecology" OR "Oncology" OR "Ophthalmology" OR "Pathology" OR "Pediatrics" OR "Pharmacology & Pharmacy" OR "Physiology" OR "Public, Environmental & Occupational Health" OR "Respiratory System" OR "Toxicology" OR "Urology & Nephrology" OR "Reproductive Biology" OR "Dermatology" OR "Allergy")) OR (WC="veterinary sciences" AND (TS="rat" OR TS="rats" OR TS="mouse" OR TS="murine" OR TS="mice" OR TS="guinea" OR TS="muridae" OR TS=rabbit* OR TS=lagomorph* OR TS=hamster* OR TS=ferret* OR TS=gerbil* OR TS=rodent* OR TS="dog" OR TS="dogs" OR TS=beagle* OR TS="canine" OR TS="cats" OR TS="feline" OR TS="pig" OR TS="pigs" OR TS="swine" OR TS="porcine" OR TS=monkey* OR TS=macaque* OR TS=baboon* OR TS=marmoset*)) OR (TS=toxic* AND (TS="rat" OR TS="rats" OR TS="mouse" OR TS="murine" OR TS="mice" OR TS="guinea" OR TS="muridae" OR TS=rabbit* OR TS=lagomorph* OR TS=hamster* OR TS=ferret* OR TS=gerbil* OR TS=rodent* OR TS="dog" OR TS="dogs" OR TS=beagle* OR TS="canine" OR TS="cats" OR TS="feline" OR TS="pig" OR TS="pigs" OR TS="swine" OR TS="porcine" OR TS=monkey* OR TS=macaque* OR TS=baboon* OR TS=marmoset* OR TS="child" OR TS="children" OR TS=adolescen* OR TS=infant* OR TS="WORKER" OR TS="HUMAN" OR TS=patient*)) OR TS="exposure")	326

Database search date	Terms	Hits
ToxLine 07/12/16	@syn0+@or+(piscesqcorrection+hexabromocyclododecane*+hbcd*+@term+@rn+3194-55-6+@term+@rn+25637-99-4)+@and+@range+yr+2014+2016+@not+@org+pubmed+pubdart+"nih+reporter"+tscats	0
	@syn0+@or+(piscesqcorrection+"Bromkal+73-6CD"+"Bromkal+73-6D"+"HBCD-LM"+"HBCD-LMS"+"HBCD-SP+75"+"Myflam+11645"+"Nicca+Fi-None+CG+1"+"Nicca+Fi-None+TS+1"+"Nicca+Fi-None+TS+3"+"Nicca+Fi-None+TS+88"+"Pyroguard+F+800"+"Pyroguard+SR+103"+"Pyroguard+SR+103A")+@and+@range+yr+2014+2016+@not+@org+pubmed+pubdart+"nih+reporter"+tscats	
	@syn0+@or+(piscesqcorrection+"Pyroguard+SR+103HR"+"Pyroguard+SR+104"+"Pyrovatex+3887"+"Safron+5261"+"Saytex+HBCD"+"Saytex+HBCD+LM"+"Saytex+HBCD+SF"+"Saytex+HP+900"+"Saytex+HP+900G")+@and+@range+yr+2014+2016+@not+@org+pubmed+pubdart+"nih+reporter"+tscats	
11/14/14	@syn0+@or+(hexabromocyclododecane*+hbcd*+@term+@rn+3194-55-6+@term+@rn+25637-99-4)+@and+@range+yr+2013+2014+@not+@org+pubmed+pubdart+"nih+reporter"	0
	@syn0+@or+("Bromkal+73-6CD"+"Bromkal+73-6D"+"HBCD-LM"+"HBCD-LMS"+"HBCD-SP+75"+"Myflam+11645"+"Nicca+Fi-None+CG+1"+"Nicca+Fi-None+TS+1"+"Nicca+Fi-None+TS+3"+"Nicca+Fi-None+TS+88"+"Pyroguard+F+800"+"Pyroguard+SR+103"+"Pyroguard+SR+103A")+@and+@range+yr+2013+2014+@not+@org+pubmed+pubdart+"nih+reporter"+tscats	
	@syn0+@or+("Pyroguard+SR+103HR"+"Pyroguard+SR+104"+"Pyrovatex+3887"+"Safron+5261"+"Saytex+HBCD"+"Saytex+HBCD-LM"+"Saytex+HBCD-SF"+"Saytex+HP+900"+"Saytex+HP+900G")+@and+@range+yr+2013+2014+@not+@org+pubmed+pubdart+"nih+reporter"+tscats	
06/09/14	@syn0+@or+("1,2,5,6,9,10-hexabromocyclodecane"+hexabromocyclododecane*+hbcd*+@term+@rn+3194-55-6+@term+@rn+25637-99-4)+@and+@range+yr+2013+2014+@not+@org+pubmed+pubdart+"nih+reporter"	0
	@syn0+@or+("Bromkal+73-6CD"+"Bromkal+73-6D"+"HBCD-LM"+"HBCD-LMS"+"HBCD-SP+75"+"Myflam+11645"+"Nicca+Fi-None+CG+1"+"Nicca+Fi-None+TS+1"+"Nicca+Fi-None+TS+3"+"Nicca+Fi-None+TS+88"+"Pyroguard+F+800"+"Pyroguard+SR+103"+"Pyroguard+SR+103A")+@and+@range+yr+2013+2014+@not+@org+pubmed+pubdart+"nih+reporter"+tscats	0
	@syn0+@or+("Pyroguard+SR+103HR"+"Pyroguard+SR+104"+"Pyrovatex+3887"+"Safron+5261"+"Saytex+HBCD"+"Saytex+HBCD-LM"+"Saytex+HBCD-SF"+"Saytex+HP+900"+"Saytex+HP+900G")+@and+@range+yr+2013+2014+@not+@org+pubmed+pubdart+"nih+reporter"+tscats	0
08/22/13	@OR+(@term+@rn+25637-99-4+@term+@rn+3194-55-6)+@NOT+@org+pubmed+pubdart+"nih+reporter"+tscats	22
	@OR+("hexabromocyclodecane"+"hexabromocyclododecane"+"hexabromocyclododecane"+"hexabromocyclododecanes"+"hbcd"+"hbcds")+@NOT+@org+pubmed+pubdart+"nih+reporter"+tscats	20

ED_005297A_00166423-00012

Database search date	Terms	Hits
TSCATS 1 07/12/16	@or+(@term+@rn+25637-99-4+@term+@rn+3194-55-6)+@and+@range+yr+2014+2016+@and+@org+tscats	0
11/14/14	@or+(@term+@rn+25637-99-4+@term+@rn+3194-55-6)+@and+@range+yr+2013+2014+@and+@org+tscats	0
06/09/14	@or+(@term+@rn+25637-99-4+@term+@rn+3194-55-6)+@and+@range+yr+2013+2014+@and+@org+tscats	0
08/22/13	@term+@rn+25637-99-4+@AND+@org+tscats	12
	@term+@rn+3194-55-6+@and+@org+tscats	53
TSCATS 2 07/12/16	[HYPERLINK "https://java.epa.gov/oppt_chemical_search/"] date limited, 11/01/2014-date of search	0
11/14/14	3194-55-6, 25637-99-4 date limited, 2014-date of search	0
06/06/14	3194-55-6, 25637-99-4 date limited, 2013-date of search	0
08/22/13	3194-55-6, 25637-99-4 date limited, 2000-date of search	10
TSCA 8e/FYI recent submissions 07/12/16	Google: 3194-55-6 25637-99-4 (8e OR fyi) tsca	0
11/14/14	Google: 3194-55-6 25637-99-4 (8e OR fyi) tsca	0
06/06/14	Google: 3194-55-6 25637-99-4 (8e OR fyi) tsca	0
08/22/13	Google: 3194-55-6 25637-99-4 (8e OR fyi) tsca	4
Combined reference set	(duplicates eliminated through electronic screen)	916

Table B-2. Processes used to augment the search of core computerized databases for HBCD

System used	Selected key reference(s) or sources	Date	Additional references identified
Manual search of citations from health assessment documents	{EINECS, 2008, 1443914@@author-year}. Risk assessment: Hexabromocyclododecane. CAS-No.: 25637-99-4. Final report. Luxembourg: European Inventory of Existing Commercial Chemical Substances, Office for Official Publications of the European Communities	9/2013	7 citations added
	{Environment Canada, 2011, 1937209@@author-year}. Screening Assessment Report on Hexabromocyclododecane; Chemical Abstracts Service Registry Number 3194-55-6, Environment Canada, Health Canada	9/2013	0 citations added
WOS, forward search	{Ema, 2008, 787657@@author-year}. Two-generation reproductive toxicity study of the flame retardant hexabromocyclododecane in rats. Reprod Toxicol 25: 335-351. http://dx.doi.org/10.1016/j.reprotox.2007.12.004	9/2013	0 citations added

ED_005297A_00166423-00013

System used	Selected key reference(s) or sources	Date	Additional references identified
	{Eriksson, 2006, 787660@@author-year}. Impaired behaviour, learning and memory, in adult mice neonatally exposed to hexabromocyclododecane (HBCDD). Environ Toxicol Pharmacol 21: 317-322. http://dx.doi.org/10.1016/j.etap.2005.10.001	9/2013	0 citations added
	{Saegusa, 2009, 787721@@author-year}. Developmental toxicity of brominated flame retardants, tetrabromobisphenol A and 1,2,5,6,9,10-hexabromocyclododecane, in rat offspring after maternal exposure from mid-gestation through lactation. Reprod Toxicol 28: 456-467. http://dx.doi.org/10.1016/j.reprotox.2009.06.011	9/2013	0 citations added
	{van der Ven, 2009, 589273@@author-year}. Endocrine effects of hexabromocyclododecane (HBCD) in a one-generation reproduction study in Wistar rats. Toxicol Lett 185: 51-62. http://dx.doi.org/10.1016/j.toxlet.2008.12.003	9/2013	0 citations added
WOS, backward search	{Ema, 2008, 787657@@author-year}. Two-generation reproductive toxicity study of the flame retardant hexabromocyclododecane in rats. Reprod Toxicol 25: 335-351. http://dx.doi.org/10.1016/j.reprotox.2007.12.004	9/2013	2 citations added
	{Eriksson, 2006, 787660@@author-year}. Impaired behaviour, learning and memory, in adult mice neonatally exposed to hexabromocyclododecane (HBCDD). Environ Toxicol Pharmacol 21: 317-322. http://dx.doi.org/10.1016/j.etap.2005.10.001	9/2013	1 citation added
	{Saegusa, 2009, 787721@@author-year}. Developmental toxicity of brominated flame retardants, tetrabromobisphenol A and 1,2,5,6,9,10-hexabromocyclododecane, in rat offspring after maternal exposure from mid-gestation through lactation. Reprod Toxicol 28: 456-467. http://dx.doi.org/10.1016/j.reprotox.2009.06.011	9/2013	0 citations added
	{van der Ven, 2009, 589273@@author-year}. Endocrine effects of hexabromocyclododecane (HBCD) in a one-generation reproduction study in Wistar rats. Toxicol Lett 185: 51-62. http://dx.doi.org/10.1016/j.toxlet.2008.12.003	9/2013	0 citations added
References obtained during the assessment process	Snowball search	9/2013, Ongoing	42 citations added
Search of online chemical assessment-related websites	Combination of CASRNs and synonyms searched on the following websites: ACGIH ([HYPERLINK "http://www.acgih.org/home.htm"]) AIHA WEELs ([HYPERLINK "http://www.tera.org/OARS/WEEL.html"]) ATSDR ([HYPERLINK "http://www.atsdr.cdc.gov/substances/index.asp"]) CalEPA Office of Environmental Health Hazard Assessment ([HYPERLINK "http://www.oehha.ca.gov/risk.html"])	7/13/2016 11/14/2014 6/9/2014 8/26/2013	4 citations added 1 citation added 1 citation added 10 citations added

ED_005297A_00166423-00014

System used	Selected key reference(s) or sources	Date	Additional references identified
	OEHHA Toxicity Criteria Database ([HYPERLINK "http://www.oehha.ca.gov/tcdb/index.asp"]) Biomonitoring California-Priority Chemicals ([HYPERLINK "http://www.oehha.ca.gov/multimedia/biomon/pdf/PriorityChemsCurrent.pdf"]) Biomonitoring California-Designated Chemicals ([HYPERLINK "http://www.oehha.ca.gov/multimedia/biomon/pdf/DesignatedChemCurrent.pdf"]) Cal/Ecotox Database ([HYPERLINK "http://www.oehha.ca.gov/scripts/cal_ecotox/CHEMLIST.ASP"]) CalEPA Drinking Water Notification Levels ([HYPERLINK "http://www.swrcb.ca.gov/drinking_water/certlic/drinkingwater/NotificationLevels.shtml"]) OEHHA Fact Sheets ([HYPERLINK "http://www.oehha.ca.gov/public_info/facts/index.html"]) Non-cancer health effects Table (RELs) ([HYPERLINK "http://www.oehha.ca.gov/air/allrels.html"]) and Cancer Potency Factors (Appendix A and AppendixB) ([HYPERLINK "http://www.oehha.ca.gov/air/hot_spots/tsd052909.html"]) CHRIP ([HYPERLINK "http://www.safe.nite.go.jp/english/db.html"]) CPSC ([HYPERLINK "http://www.cpsc.gov"]) ECETOC publications ([HYPERLINK "http://www.ecetoc.org/publications"]) ECHA General site ([HYPERLINK "http://echa.europa.eu/information-on-chemicals"]) ECHA info on Registered Substances ([HYPERLINK "http://echa.europa.eu/information-on-chemicals/registered-substances"]) ECHA Information from the Existing Substances Regulation (ESR) ([HYPERLINK "http://echa.europa.eu/information-on-chemicals/information-from-existing-substances-regulation"]) eChemPortal (participating databases: ACToR, AGRITOX, CCR, CCR DATA, CESAR, CHRIP, ECHA CHEM, EnviChem, ESIS, GHS-J, HPVIS, HSDB, HSNO CCID, INCHEM, J-CHECK, JECDB, NICNAS PEC, OECD HPV, OECD SIDS IUCLID, SIDS UNEP, UK CCRMP Outputs, US EPA IRIS, US EPA SRS) ([HYPERLINK "http://www.echemportal.org/echemportal/participant/page.action?pageID=9"]) Environment Canada – Search entire site ([HYPERLINK "http://www.ec.gc.ca/default.asp?lang=En&n=ECD35C36"]) if not found below: Toxic Substances Managed Under CEPA ([HYPERLINK "http://www.ec.gc.ca/toxiques-toxics/Default.asp?lang=En&n=98E80CC6-1"]) Search results		

ED_005297A_00166423-00015

System used	Selected key reference(s) or sources	Date	Additional references identified
	Final Assessments ([HYPERLINK "http://www.ec.gc.ca/lcpe-cepa/default.asp?lang=En&xml=09F567A7-B1EE-1FEE-73DB-8AE6C1EB7658"]) Draft Assessments ([HYPERLINK "http://www.ec.gc.ca/lcpe-cepa/default.asp?lang=En&xml=6892C255-5597-C162-95FC-4B905320F8C9"]) EPA CDAT ([HYPERLINK "http://java.epa.gov/oppt_chemical_search/"]) EPA Acute Exposure Guideline Levels ([HYPERLINK "http://www.epa.gov/oppt/aegl/pubs/chemlist.htm"]) EPA NSCEP ([HYPERLINK "http://www.epa.gov/ncepihom/"]) EPA OPP ([HYPERLINK "http://iaspub.epa.gov/apex/pesticides/f?p=chemicalsearch:1"]) EPA Science Inventory ([HYPERLINK "http://cfpub.epa.gov/si/"]) ERPGs ([HYPERLINK "https://www.aiha.org/get-involved/AIHAGuidelineFoundation/EmergencyResponsePlanningGuidelines/Pages/default.aspx"]) FDA ([HYPERLINK "http://www.fda.gov/"]) Federal Docket ([HYPERLINK "file:///C:/Users/stickney.ESC1/AppData/riccardi/AppData/Local/Microsoft/AppData/Local/IRIS%20Tox%20Reviews/RDX/Search History/LSP_201X/FOR%20INTERNAL%20USE%20ONLY%20-%20Search%20Table/www.regulations.gov"]) Health Canada – Search entire site ([HYPERLINK "http://www.hc-sc.gc.ca/index-eng.php"]) Health Canada Drinking Water Documents ([HYPERLINK "http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/index-eng.php" \l "tech_doc"]) Health Canada First Priority List Assessments ([HYPERLINK "http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl1-lsp1/index-eng.php"]) Health Canada Second Priority List Assessments ([HYPERLINK "http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl2-lsp2/index-eng.php"]) IARC Index: ([HYPERLINK "http://monographs.iarc.fr/ENG/Monographs/vol101/mono101-B02-B03.pdf"]) IRISTrack/New Assessments and Reviews ([HYPERLINK "http://cfpub.epa.gov/ncea/iris/search/"]) Japan Existing Chemical Data Base (JECDB) ([HYPERLINK "http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp"]) NAP – Search Site ([HYPERLINK "http://www.nap.edu/"]) NCI ([HYPERLINK "http://www.cancer.gov"]) National Center for Toxicological Research ([HYPERLINK "http://www.fda.gov/AboutFDA/CentersOffices/OC/OfficeofScientificandMedicalPrograms/NCTR/default.htm"])		

System used	Selected key reference(s) or sources	Date	Additional references identified
	NICNAS (PEC only covered by eChemPortal) ([HYPERLINK "http://www.nicnas.gov.au/industry/aics/search.asp"]) NIEHS ([HYPERLINK "http://www.niehs.nih.gov/"]) NIOSH ([HYPERLINK "http://www.cdc.gov/niosh/topics/"]) NIOSHTIC 2 ([HYPERLINK "http://www2a.cdc.gov/nioshtic-2/"]) NTP - RoC, status, results, and management reports 12th Report On Carcinogens: ([HYPERLINK "http://ntp.niehs.nih.gov/?objectid=03C9AF75-E1BF-FF40-DBA9EC0928DF8B15"]) 13th Report On Carcinogens: ([HYPERLINK "http://ntp.niehs.nih.gov/?objectid=03C9AF75-E1BF-FF40-DBA9EC0928DF8B15"]) NTP Site Search: ([HYPERLINK "http://ntpsearch.niehs.nih.gov/texis/search/?query=arsenic&pr=ntp_web_entire_site_all&mu=Entire+NTP+Site"]) OECD HPV/SIDS/IUCLID (cross-check with eChem) ([HYPERLINK "http://webnet.oecd.org/hpv/ui/Search.aspx"]) OSHA ([HYPERLINK "http://www.osha.gov/dts/chemicalsampling/toc/toc_chemsamp.html"]) RTECS ([HYPERLINK "http://www.ccohs.ca/search.html"]) UNEP SIDS (through 2007) ([HYPERLINK "http://www.chem.unep.ch/irptc/sids/OECDSIDS/sidspub.html"])		

ACGIH = American Conference of Governmental Industrial Hygienists; ACToR = Aggregated Computational Toxicology Resource; AIHA = American Industrial Hygiene Association; ATSDR = Agency for Toxic Substances and Disease Registry; CalEPA = California Environmental Protection Agency; CASRN = Chemical Abstracts Service Registry Number; CCID = Chemical Classification Information Database; CCR = Canadian Categorization Results; CCRMP = Coordinated Chemicals Risk Management Programme Publications; CDAT = Chemical Data Access Tool; CEPA = Canadian Environmental Protection Act; CESAR = Canada's Existing Substances Assessment Repository; CHRIP = Chemical Risk Information Platform; CPSC = Consumer Product Safety Commission; ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; ECHA = European Chemicals Agency; EnviChem = Data Bank of Environmental Properties of Chemicals; EPA = Environmental Protection Agency; ERPG = Emergency Response Planning Guidelines; ESIS = European chemical Substances Information System; FDA = Food and Drug Administration; GHS-J = Globally Harmonized System-Japan; HPV = High Production Volume; HPVIS = High Production Volume Information System; HSDB = Hazardous Substances Data Bank; HSNO = Hazardous Substances and New Organisms; IARC = International Agency for Research on Cancer; IRIS = Integrated Risk Information System; IUCLID = International Uniform Chemical Information Database; J-CHECK = Japan CHEmicals Collaborative Knowledge; JECDB = Japan Existing Chemical Data Base; NAP = National Academies Press; NAS = National Academy of Sciences; NCI = National Cancer Institute; NICNAS = National Industrial Chemicals Notification and Assessment Scheme; NIEHS = National Institute for Environmental Health Sciences; NIOSH = National Institute for Occupational Safety and Health; NIOSHTIC = National Institute for Occupational Safety and Health Technical Information Center; NRC = National Research Council; NSCEP = National Service Center for Environmental Publications; NTP = National Toxicology Program; OECD = Organisation for Economic Cooperation and Development; OEHHA = Office of Environmental Health Hazard Assessment; OPP = Office of Pesticide Programs; OSHA = Occupational Safety and Health Administration; PEC = Priority Existing Chemical; REL = Reference Exposure Level; RoC = Report on Carcinogens; RTECS = Registry of Toxic Effects of Chemical Substances;

ED_005297A_00166423-00017

SIDS = Screening Information Data Set; SRS = Substance Registry Services; UK = United Kingdom; UNEP = United Nations Environment Programme; WEEL = Workplace Environmental Exposure Level

B.2 DETAILS OF THE EVALUATION OF EPIDEMIOLOGY STUDIES

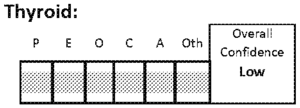
Commented [RS3]: We anticipate that the documentation of study quality for epidemiology studies included in Table B-3 will be revised or replaced with the risk of bias evaluations currently being performed HAWC.

The evaluation of the epidemiology studies of HBCD considered aspects of the study design affecting the internal or external validity of the results (e.g., population characteristics and representativeness, exposure and outcome measures, confounding, data analysis). This evaluation focused on specific types of bias (e.g., selection bias, information bias due to exposure misclassification), aspects of the sensitivity of the design and analysis that could affect the ability of the study to detect a true hazard, and other considerations that could otherwise influence or limit the interpretation of the data. Documentation of the evaluation of individual studies is provided in Table B-3.

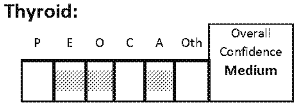
1 **Table B-3. Summary of evaluation of epidemiologic studies of HBCD**

Study, population	Exposure measure and range	Outcome measure	Confounding	Statistical methods and presentation of results	Confidence[a]
{Eggesbø, 2011, 787656@@author-year} (Norway, 2003–2006) Birth cohort Infants (n = 193)	Breast milk Total HBCD LOQ 0.2 ng/g lipid Median 0.54 ng/g lipid Range 0.13–31 ng/g lipid 32% less than the LOD (0.1 ng/g lipid; used as referent category in the categorical analysis)	TSH (data from clinical lab screening for congenital hypothyroidism)	Adjusted for age at TSH screening, maternal BMI, county, p,p'-DDE, hexachlorobenzene, delivery type, pregnancy preeclampsia, and hypertension. Also evaluated maternal education, age at delivery, Norwegian nationality, season, parity, smoking, sex, gestational age, beta-hexachlorocylohexane, oxychlordane, and sum of all PCB congeners.	Categorical HBCD (32% less than the LOD used as referent group), with remaining samples divided by quartile (lower confidence in analyses of HBCD as continuous measure). Analysis of TSH as continuous variable (lnTSH) and dichotomized at >80th percentile. Lipid-adjusted HBCD.	**Thyroid:** [EMBED PBrush] No details of TSH analysis provided (other than use of screening laboratory)
{Johnson, 2013, 1676758@@author-year} (United States, 2002–2003) Adult men (infertility clinic) (n = 38)	Household dust Total HBCD LOD not reported Median 246 ng/g dust 90th percentile 1,103 ng/g dust 3% less than the LOD	Thyroid hormones; details of analysis (coefficient of variation, LOD) provided in {Meeker, 2008, 2238550@@author-year}	Considered adjustment for age and BMI; limited to men.	Spearman correlation (continuous HBCD) HBCD measured in dust (lipid-adjustment not applicable). Results reported only as absence of statistical significance.	**Thyroid and steroidal/ gonadotropin hormones:** [EMBED PBrush] Limited analysis and inadequate reporting of results; small sample size

ED_005297A_00166423-00019

Study, population	Exposure measure and range	Outcome measure	Confounding	Statistical methods and presentation of results	Confidence[a]
		Steroidal and gonadotropin hormones; details of analysis (coefficient of variation, LOD) provided in {Meeker, 2008, 2238550@@author-year}	Adjusted for age and BMI; limited to men.	Results for outcomes other than testosterone and SHBG reported only as absence of statistical significance.	
{Kim, 2014, 2324769@@author-year} (South Korea, 2009–2010) Infants with congenital hypothyroidism (26 cases, 12 controls)	Serum (maternal and infant's) Total HBCD (and individual stereoisomers) LOQ 0.036 ng/g lipid Mean 8.55 ng/g lipid Range <LOQ–166 ng/g lipid Percent less than the LOQ not reported (EPA estimates from figure to be ≥25%)	Congenital hypothyroidism (case definition not reported)	No adjustment age of mother (mean 33 yrs) or baby (most 1–3 mo) but these factors did not differ between cases and controls); sex of babies not reported. Excluded obese mothers; only for normal group mothers (criterion not defined).	t-Test on normalized distribution, with outliers (undefined) excluded. Lipid-adjusted HBCD. Percent less than the LOQ not reported (imputed values).	**Thyroid:**  No information on recruitment process for cases or controls; 2 of the 26 cases were ages 18 and 24 mo; approximately 25% less than the LOD; uncertain impact of exclusion of outliers

ED_005297A_00166423-00020

Study, population	Exposure measure and range	Outcome measure	Confounding	Statistical methods and presentation of results	Confidence[a]
{Kiciński, 2012, 1927571@@author-year} (Belgium, 2008–2011) Ages 13–17 (n = 515)	Serum (child's) Total HBCD LOQ 30 ng/L Median less than the LOQ (30 ng/L) Range <30–234 ng/L >75% less than the LOQ	Thyroid: no details of thyroid hormone analysis provided Neurodevelopment: standard tests for motor function, cognition, attention; references provided	Adjusted for age, gender, blood lipids, BMI. Additional covariates evaluated included smoking, parental smoking, parental education, and parental home ownership, physical activity, computer use, alcohol and fish consumption, blood lead and blood PCBs, and type of education (child), and were included based on a stepwise regression procedure.	Regression models HBCD dichotomized as above versus below LOQ. Analysis of hormones as continuous variables. Lipids included in model. >75% of samples were less than the LOQ.	**Thyroid:**  No information on thyroid hormone assays; 75% of HBCD less than the LOD (dichotomized analysis) **Neurodevelopment:** [EMBED PBrush] Exposure measure does not adequately represent relevant time window of exposure for neurodevelopmental outcomes; 75% of HBCD less than the LOD (dichotomized analysis)
{Meijer, 2012, 1401499@@author-year} Birth cohort Age 3 mo (n = 34)	Serum (maternal) Total HBCD LOQ 0.9 pg/g serum Median 0.7 ng/g lipid Range (<LOD–7.4) ng/g lipid 2% less than the LOD	Steroidal and gonadotropin hormones; details provided in {Laven, 2004, 2238548@@author-year}	Limited age range, limited to boys; no discussion of consideration of confounders.	Spearman correlation (continuous HBCD). Lipid-adjusted HBCD. Results for outcomes other than testosterone reported only as absence of statistical significance.	**Steroidal/gonadotropin hormones:** [EMBED PBrush] Limited analysis and inadequate reporting of results; small sample size

ED_005297A_00166423-00021

Study, population	Exposure measure and range	Outcome measure	Confounding	Statistical methods and presentation of results	Confidence[a]
{Roze, 2009, 758049@@author-year} (the Netherlands, 2001–2002 at baseline) Birth cohort Infants (n = 51)	Serum (maternal) Total HBCD LOQ 0.8 pg/g serum Median 0.8 ng/g lipid Range 0.3–7.5 ng/g lipid 0% less than the LOD	Thyroid: No details of thyroid hormone analysis (measured in cord blood samples)	Limited age range (5 yrs 8 mo to 6 yrs 2 mo); no discussion of consideration of confounders.	Spearman correlation (continuous HBCD). Lipid-adjusted HBCD. Results reported only as absence of statistical significance.	**Thyroid:** [EMBED PBrush] No information on thyroid hormone assays; limited analysis and inadequate reporting of results; small sample size
		Neurodevelopment: standard tests for motor function, cognition, attention, and hyperactivity (references provided)	Limited age range (5 yrs 8 mo to 6 yrs 2 mo); adjusted for maternal education, home environment score, sex.	Spearman correlation (continuous HBCD) Lipid-adjusted HBCD. Results for tests other than coordination, verbal and total intelligence reported only as absence of statistical significance.	**Neurodevelopment:** [EMBED PBrush] Limited analyses and inadequate reporting of results; small sample size

[a]Evaluation of sources of bias or study limitations (see Toxicological Review, Systematic Review Methods, Considerations for Evaluation of Epidemiology Studies): P = population selection; E = exposure misclassification; O = outcome misclassification; C = confounding; A = analysis; Oth = other feature affecting interpretation of results. Extent of column shading reflects degree of limitation.

BMI = body mass index; LOD = limit of detection; LOQ = limit of quantitation; PCB = polychlorinated biphenyl; SHBG = sex hormone binding globulin; TSH = thyroid-stimulating hormone

ED_005297A_00166423-00022

B.3 DETAILS OF THE EVALUATION OF EXPERIMENTAL ANIMAL STUDIES

Commented [RS4]: We anticipate that the documentation of study quality for experimental animal studies included in Table B-4 will be revised or replaced with the risk of bias evaluations currently being performed HAWC.

The evaluation of the experimental animal studies of HBCD examined aspects of five methodological features of toxicity studies (i.e., test animal, experimental design, exposure, endpoint evaluation, and results presentation). Some methodological features (e.g., exposure) are likely to be relatively independent of the outcome examined by the study while others (e.g., endpoint evaluation) are more outcome specific. Documentation of the evaluation of individual studies is provided in Table B-4.

ED_005297A_00166423-00023

Table B-4. Summary of evaluation of experimental animal studies of HBCD

Test animal	Experimental design and exposure information	Endpoint evaluation	Results presentation	Conclusion
{Ema, 2008, 787657@@author-year}				
Male and female Sprague-Dawley (CRL:CD(SD)) rats obtained from Charles River, Japan Strain selected because they are commonly used for reproductive and developmental studies	Investigated multiple health effects (**thyroid, liver, female reproductive, male reproductive, developmental, nervous system, immune system**) in a two-generation reproductive toxicity study Followed OECD guidelines for a two-generation reproductive study and GLP principles F0 – 10 wks exposure prior to mating through necropsy F1/F2 offspring – maternal exposure throughout gestation/lactation F1 adults – dietary exposure post weaning until necropsy Litter size adjusted to eight pups (four males, four females) on PND 4 Test article purity (99.7%) and composition (8.5% alpha, 7.9% beta, and 83.7% gamma) reported Dietary; HBCD mixed into powdered diet (no vehicle); homogeneity and stability in feed analyzed; dose administered in diet evaluated Included concurrent control Received standard diet and water ad libitum Design and exposure determined to be suitable for investigating all endpoints planned for in the study	Methodology acceptable and adequately described for all endpoints, unless listed separately below. <u>*Nervous system*</u> Blinding of scorer not reported for FOB, executive function, and locomotor activity. *Note: potential for observer bias is expected to be low for locomotor activity and executive function due to use of automated scoring/ limited observer interaction.* <u>*Immune system*</u> Measured only observational endpoints, which are less sensitive measures of immunotoxicity.	Thorough presentation of quantitative data, experimental unit, and sample size in text/figures/tables for all endpoints.	Design of the study was determined to be suitable for investigating multiple endpoints representing various health hazard domains across multiple generations and lifestages. Study conduct and reporting were determined acceptable, unless concerns are noted in the 'Endpoint evaluation' and 'Results presentation' columns to the left. **High confidence**: *Thyroid* *Liver* *Female reproductive* *Male reproductive* *Developmental* **Medium confidence**: *Nervous system* *Immune system*

Test animal	Experimental design and exposure information	Endpoint evaluation	Results presentation	Conclusion
{Lilienthal, 2009, 787693@@author-year}				
Male and female Wistar (HsdCpb:WU) rats obtained from RIVM	Investigated **nervous system** effects in a 1-generation reproductive study Followed OECD guidelines for a 1-generation reproductive study; except distributed animals across more dose groups with fewer animals (i.e., 5/sex/dose). Design and exposure chosen to investigate the dose-response trend using BMD modeling software. F0 – 10 or 2 wks exposure prior to mating in males and females, respectively F1 – continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning until sacrifice (~PNW 20) Litter size was not standardized Test article purity was not reported (trace tetra- and pentabromocyclododecane noted); composition (10.3% alpha, 8.7% beta, and 81.0% gamma) reported Dietary; corn oil vehicle (first dissolved in acetone; allowed to evaporate) Included concurrent control Internal dosing verified by analysis of isomers in liver Received soy-free diet and water ad libitum Used eight exposure groups (including control) with low, incremental doses (i.e., 0.1, 0.3, 1, 3, 10, 30, 100). Only 3–5 rats/sex/dose were investigated for each endpoint. The selected doses and small sample sizes have the potential to limit the ability to detect significant differences between the dose groups, especially for endpoints with higher expected variability.	Methodology acceptable and adequately described for all endpoints, unless listed separately below. *Catalepsy* Unclear whether animals received rest period between all poses.	Thorough presentation of quantitative data, experimental unit, and sample size in text/figures/tables for all endpoints.	The study was designed to investigate dose-response trends; however, some concern exists around the use of small sample sizes for investigating exposure-related effects. Conduct and reporting of the study was determined to be suitable, unless concerns are noted in the 'Endpoint evaluation' and 'Results presentation' columns to the left. **Medium confidence**: *Nervous system*

Test animal	Experimental design and exposure information	Endpoint evaluation	Results presentation	Conclusion
{van der Ven, 2009, 589273@@author-year}				
Male and female Wistar rats obtained from RIVM	Investigated multiple health effects **(thyroid, liver, female reproductive, male reproductive, developmental, nervous system, immune system)** in a 1-generation reproductive study Followed OECD guidelines for a 1-generation reproductive study; except distributed animals across more dose groups with fewer animas (i.e., 5/sex/dose). Design and exposure chosen to investigate the dose-response trend using BMD modeling software. F0 – 10 or 2 wks exposure prior to mating in males and females, respectively F1 – continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning until sacrifice (~PNW 20) Litter size was not standardized Test article purity was not reported (trace tetra- and pentabromocyclododecane noted); composition (10.3% alpha, 8.7% beta, and 81.0% gamma) reported Dietary; corn oil vehicle (first dissolved in acetone; allowed to evaporate) Included concurrent control Internal dosing verified by analysis of isomers in liver Received soy-free diet and water ad libitum Study used eight exposure groups (including control) with low, incremental doses (i.e., 0.1, 0.3, 1, 3, 10, 30, 100). Only 3–5 rats/sex/dose were investigated for each endpoint. The selected doses and small sample sizes have the potential to limit the ability to detect significant differences between the	Methodology acceptable and adequately described for all endpoints, unless listed separately below. *Nervous system* Only evaluated brain weight, which is an insensitive measure of neurotoxicity.	Thorough presentation of quantitative data, experimental unit, and sample size in text/figures/tables for all endpoints, unless listed separately below. *AGD in F1 males* Sample size unclear. *Pup body weight* Experimental unit and sample size unclear.	The study was designed to investigate dose-response trends; however, some concern exists around the use of small sample sizes for investigating exposure-related effects. Conduct and reporting of the study was determined to be suitable, unless concerns are noted in the 'Endpoint evaluation' and 'Results presentation' columns to the left. **Medium confidence:** *Thyroid* *Liver* *Female reproductive* *Male reproductive* *Developmental* *Immune system* **Low confidence:** *Nervous system*

Test animal	Experimental design and exposure information	Endpoint evaluation	Results presentation	Conclusion
	dose groups, especially for endpoints with high expected variability (e.g., thyroid hormones, immunological endpoints).			
{WIL Research, 2001, 787787@@author-year} Reference is also known as: Chengelis CP, A 90-day oral (gavage) toxicity study of HBCD in rats, WIL Research Laboratories, Inc., Ashland, Ohio, USA, 2001. Peer reviewed by Versar, Inc. for EPA in 2014; determined to provide useful information on the toxicity of HBCD.				
Male and female Sprague-Dawley (CRL:CD(SD)IGD BR) rats obtained from Charles River, USA	Investigated multiple health effects **(thyroid, liver, female reproductive, male reproductive, nervous system)** in a 90-d study [followed by a 28-d recovery period] Followed OECD guidelines for testing health effects of chemicals and GLP principles Test article was a composite of three commercial mixtures, in equal parts, from Albemarle Corporation, Dead Sea Bromine Group/Bromine Compound LTD, and Great Lakes Corporation. Purity not reported. Composition (~6% alpha, ~5% beta, ~85% gamma) reported. Isomeric concentrations determined in adipose tissue after achieving steady stay were reported (65–70% alpha, 9–15% beta, 14–20% gamma). Daily gavage; corn oil vehicle Included concurrent control Homogeneity, stability, and concentrations of prepared doses were stated to be analyzed Received standard diet and water ad libitum Design and exposure determined to be suitable for investigating all endpoints planned for in the study	Methodology acceptable and adequately described for all endpoints, unless listed separately below. *Thyroid* TSH level in the control group was 1–2 orders of magnitude lower than reported for other studies and had a high incidence of samples <LOD. *Nervous system* Investigated FOB, locomotor activity, brain weight and gross histopathology. Brain weight and gross histopathology are insensitive measures of neurotoxicity.	Thorough presentation of quantitative data, experimental unit, and sample size in text/figures/tables for all endpoints.	Design of the study was determined to be suitable for investigating multiple endpoints representing various health hazard domains following a 90-day exposure. Conduct and reporting of the study was also determined acceptable, unless concerns are noted in the 'Endpoint evaluation' and 'Results presentation' columns to the left. **High confidence:** *Liver* *Female reproductive* *Male reproductive* **Medium confidence:** *Thyroid* *Nervous system*
{van der Ven, 2006, 787745@@author-year}				
Male and female Wistar (RIVM	Investigated multiple health effects **(thyroid, liver, female reproductive, male reproductive, nervous system, immune**	Methodology acceptable and adequately	Thorough presentation of	The study was designed to investigate dose-

Test animal	Experimental design and exposure information	Endpoint evaluation	Results presentation	Conclusion
Cpb:WU) rats obtained from RIVM	**system)** in a 28-d study Followed OECD guidelines for 28-d subacute toxicity testing, except distributed animals across more dose groups with fewer animas (i.e., 5/sex/dose). Design and exposure chosen to investigate the dose-response trend using BMD modeling software. Test article purity not reported (trace tetra- and pentabromocyclododecane noted); composition (10.3% alpha, 8.7% beta and 81.0% gamma) reported Daily gavage; corn oil vehicle Included concurrent control Internal dosing verified by analysis of isomers in liver and fat Received soy-free diet and water ad libitum Study used nine exposure groups (including control) with low, incremental doses (i.e., 0.1, 0.3, 1, 3, 10, 30, 100, 200). Only 3–5 rats/sex/dose were investigated for each endpoint. The selected doses and small sample sizes have the potential to limit the ability to detect significant differences between the dose groups, especially for endpoints with high expected variability (e.g., thyroid hormones, immunological endpoints).	described for all endpoints, unless listed separately below. _Nervous system_ Only evaluated brain weight, which is an insensitive measure of neurotoxicity.	quantitative data, experimental unit, and sample size in text/figures/tables for all endpoints, unless listed separately below. _Thyroid_ Quantitative histopathologic data not reported.	response trends; however, some concern exists around the use of small sample sizes for investigating exposure-related effects. Conduct and reporting of the study was determined to be suitable, unless concerns are noted in the 'Endpoint evaluation' and 'Results presentation' columns to the left. **Medium confidence:** *Thyroid* *Liver* *Female reproductive* *Male reproductive* *Immune system* **Low confidence:** *Nervous system*
{WIL Research, 1997, 787758@@author-year} Reference is also known as: Chengelis CP, A 28-day oral (gavage) toxicity study of HBCD in rats, WIL Research Laboratories, Inc., Ashland, Ohio, USA, 2001. Peer reviewed by Versar, Inc. for EPA in 2014; determined to provide useful information on the toxicity of HBCD.				
Male and female Sprague-Dawley (CRL:CD(SD) BR) rats obtained from Charles River, USA	Investigated multiple health effects **(thyroid, liver, nervous system)** in a 28-d study (followed by a 14-day recovery period) Followed OECD guidelines for testing health effects of chemicals and GLP principles	Methodology acceptable and adequately described for all endpoints, unless listed separately below.	Thorough presentation of quantitative data, experimental unit, and sample size in text/figures/tables	Design of the study was determined to be suitable for investigating multiple endpoints representing various health hazard

Test animal	Experimental design and exposure information	Endpoint evaluation	Results presentation	Conclusion
	Test article was a composite of equal parts of commercial mixtures from Chemical Manufacturer's Associate Brominated Flame Retardant Industry Panel members. Purity, composition, and stability were not reported. Daily gavage; corn oil vehicle Included concurrent control Homogeneity and concentrations of prepared doses were stated to be analyzed Received standard diet and water ad libitum Design and exposure determined to be suitable for investigating all endpoints planned for in the study	*Nervous system* Investigated FOB, locomotor activity, brain weight and gross histopathology. Scoring criteria were not available for FOB. Brain weight and gross histopathology are insensitive measures of neurotoxicity.	for all endpoints.	domains following a 28-day exposure. Conduct and reporting of the study was also determined acceptable, unless concerns are noted in the 'Endpoint evaluation' and 'Results presentation' columns to the left. **High confidence:** *Thyroid* *Liver* **Medium confidence:** *Nervous system*
{Hachisuka, 2010, 2919532@@author-year} Japanese publication translated into English by Apex Translation, Inc. for EPA in 2015.				
Male and female Sprague-Dawley (SD:IGS) rats; information on source of animals not provided	Investigated **developmental** and **immune system** effects in a developmental study that used maternal exposure from GD 10 to PND 20, followed by an 8-wk non-exposure period for the offspring through PNW 11 Information on the test article was not reported Dietary Included a concurrent control Study had limited reporting on aspects of design and exposure but, with the information provided, it was determined to be suitable for evaluating all endpoints investigated	Limited reporting on methodology.	The original copy of the reference was of poor quality, making it sometimes difficult to discern data reported in the tables and figures.	Limited reporting of study details affected the ability to ascertain the quality of the design and conduct. **Low confidence:** *Developmental* *Immune system*

ED_005297A_00166423-00029

Test animal	Experimental design and exposure information	Endpoint evaluation	Results presentation	Conclusion
	Due to similarities in experimental design and exposure information, it was assumed that {Hachisuka, 2010, 2919532@@author-year} and {Saegusa, 2009, 787721@@author-year} used the same cohort of animals for their experiments. For this reason, the more complete dosing information from {Saegusa, 2009, 787721@@author-year} was assumed to apply to both studies. EPA ~~An~~ attempted to contact the authors to verify the assumption that they used the same cohort of animals~~; was made but EPA received~~ no reply was received.			
{Saegusa, 2009, 787721@@author-year}				
Male and female Sprague-Dawley (Crj:CD(SD)IGS) rats obtained from Charles River, Japan	Investigated multiple health effects **(thyroid, liver, female reproductive, male reproductive, developmental, and nervous system)** in a developmental study that used maternal exposure from GD 10 to PND 20, followed by an 8-wk non-exposure period for the offspring through PNW 11 Litter size adjusted to eight pups (four males, four females) on PND 2 Animal protocol was reviewed and approved by the Animal Care and Use Committee of the National Institute of Health Science, Japan Test article purity (>95%) reported but not stability or isomeric composition Dietary exposure; unclear what, if any, vehicle was used Confirmation of doses not reported Included concurrent control Dams received a soy-free diet while offspring received a standard diet; both had water ad libitum	Methodology acceptable and adequately described for all endpoints, unless listed separately below. *Nervous system* Only evaluated brain weight, which is an insensitive measure of neurotoxicity.	Thorough presentation of quantitative data, experimental unit, and sample size in text/figures/tables for all endpoints, unless listed separately below. *Thyroid* Quantitative histopathological data not reported for offspring.	Design of the study was determined to be suitable for investigating multiple endpoints representing various health hazard domains following a developmental exposure (GD 10–PND 20). Study conduct and reporting determined acceptable, unless concerns are noted in the 'Endpoint evaluation' and 'Results presentation' columns to the left. **High confidence:** *Thyroid* *Liver* *Female reproductive* *Male reproductive*

Test animal	Experimental design and exposure information	Endpoint evaluation	Results presentation	Conclusion
	Design and exposure determined to be suitable for investigating all endpoints planned for in the study			*Developmental* **Medium confidence:** *Nervous system*
{Miller-Rhodes, 2014, 2528337@@author-year}				
Male and female Long-Evans rats obtained from Harlan Laboratories	Investigated **nervous system** effects in a developmental study using maternal exposure throughout gestation Litter size adjusted to eight pups (four males, four females) on PND 3 Animal procedures complied with approved institutional animal care protocols and were in accordance with National Institutes of Health guidelines. Test article purity (>95%) reported but not stability or isomeric composition Daily gavage; corn oil vehicle (first dissolved in acetone; allowed to evaporate overnight) Confirmation of the doses was not reported Included concurrent control Received standard diet and water ad libitum Design and exposure determined to be suitable for investigating all endpoints planned for in the study	Methodology acceptable and adequately described for all endpoints, unless listed separately below. *<u>Executive function</u>* Animals from litters showing symptoms of paralysis removed from analyses; unclear whether this was applied only to the go/no-go task or both the go/no-go and random ratio tasks. Affected animals not showing overt health effects may have been included in other analyses. Blinding of scorer not reported for grip strength measures, executive function, and locomotor activity. *Note: potential for observer bias is expected*	Thorough presentation of quantitative data, experimental unit, and sample size in text/figures/tables for all endpoints	Design of the study was determined to be suitable for investigating nervous system effects following developmental exposure (gestation). Concerns regarding conduct and reporting of the are noted in the 'Endpoint evaluation' and 'Results presentation' columns to the left. **Low confidence:** *Nervous system*

Test animal	Experimental design and exposure information	Endpoint evaluation	Results presentation	Conclusion
		to be low for executive function and locomotor activity due to use of automated scoring/ limited observer interaction.		
{Eriksson, 2006, 787660@@author-year}				
Male NMRI mice obtained from B&K, Sweden	Investigated **nervous system** effects in a developmental study using a single dose on PND 10 (i.e., time of postnatal brain growth spurt) Litter size adjusted to 10–12 pups (males and females) by PND 2 Test article purity (>98%) reported but not stability or isomeric composition Single dose gavage; HBCD suspended in egg lecithin and peanut oil (1:10) Confirmation of the doses was not reported Included concurrent control Received standard diet and water ad libitum Design and exposure determined to be suitable for investigating all endpoints planned for in the study	Methodology acceptable and adequately described for all endpoints, unless listed separately below. <u>*All endpoints*</u> Blinding of scorer not reported. <u>*Executive function*</u> External visual cues not described; unclear whether impaired visual acuity was evaluated as a possible confounder. *Note: potential for observer bias is expected to be low for locomotor activity due to use of automated scoring/ limited observer interaction.*	Thorough presentation of quantitative data, experimental unit, and sample size in text/figures/tables for all endpoints, unless listed separately below. <u>*Swim maze*</u> SD/SE not provided.	Design of the study was determined to be suitable for investigating nervous system effects following developmental exposure (PND 10). Concerns regarding conduct and reporting of the are noted in the 'Endpoint evaluation' and 'Results presentation' columns to the left. **Medium confidence:** *Nervous system*
{Yanagisawa, 2014, 2343717@@author-year}				
Male C57BL/6 mice	Investigated **liver** effects in a 105-d study using both a	Methodology acceptable	Thorough	Design, conduct and

ED_005297A_00166423-00032

Test animal	Experimental design and exposure information	Endpoint evaluation	Results presentation	Conclusion
obtained from Japan Clea Co.	standard diet and a high-fat diet Test article purity, stability and isomeric composition not reported. Weekly gavage; olive oil vehicle (first dissolved in acetone) Confirmation of the doses was not reported Included concurrent control Received standard diet and water ad libitum Study used a standard diet and high-fat diet (created by mixing lard into feed) to examine the influence of HBCD exposure on metabolic function. Doses used were several orders of magnitude lower (i.e., 0.00175–0.7 mg/kg-wk) than other HBCD studies. Concerns about the ability to discern exposure-related effects due to the low doses used. Potential confounding from the source of dietary fat, i.e., lard.	and adequately described for all endpoints.	presentation of quantitative data, experimental unit, and sample size in text/figures/tables for all endpoints, unless listed separately below. *Histopathology* Quantitative data not reported.	reporting of the study determined to be suitable, with the exception of dose selection (i.e., too low to elicit effects). High-fat arm: concern about confounding introduced by high lard content of diet. **Medium confidence:** *Liver*
{Genskow, 2015, 2919804@@author-year}				
Male C57BL/6J mice obtained from Charles River, USA	Investigated **nervous system** effects (i.e., neurochemistry) in a 30-d study (followed by a 28-d recovery period) Procedures conducted in accordance with the Guide for Care and Use of Laboratory Animals (National Institutes of Health) and approved by the Institutional Animal Care and Use Committee at Emory University. Test article purity, stability, and isomeric composition not reported Daily gavage; corn oil vehicle Confirmation of doses was not reported Included concurrent control	Methodology acceptable and adequately described for measuring neurochemistry (i.e., only nervous system effect investigated).	Thorough presentation of quantitative data, experimental unit, and sample size in text/figures/tables.	Design, conduction and reporting of the study was determined to be suitable for investigating nervous system effects following a 30-day exposure. Single-dose design did not allow examination of dose-response. **Medium confidence:** *Nervous system*

Test animal	Experimental design and exposure information	Endpoint evaluation	Results presentation	Conclusion
	Received standard diet and water ad libitum Design and exposure determined to be suitable for investigating all endpoints planned for in the study			
{Maranghi, 2013, 1927558@@author-year}				
Female BALB/c mice obtained from Charles River, USA	Investigated multiple health effects **(thyroid, liver, female reproductive, developmental)** in a 28-d study, using a single dose Test article purity, stability and isomeric composition not reported Dietary; DMSO vehicle Confirmation of the doses was not reported Included concurrent control Received standard diet altered with salmon as the main protein and fat source (to mimic human exposure) and water ad libitum Design and exposure determined to be suitable for investigating all endpoints planned for in the study	Methodology acceptable and adequately described for all endpoints.	Thorough presentation of quantitative data, experimental unit, and sample size in text/figures/tables for all endpoints, unless listed separately below. *Thyroid* Quantitative histopathological data not reported for all histological measures (i.e., follicular height).	Design of the study was determined to be suitable for investigating multiple endpoints. Concerns about the use of a nonstandard mouse diet (i.e., salmon). Single-dose design did not allow examination of dose-response. Conduct and reporting of the study was determined acceptable, unless concerns are noted in the 'Endpoint evaluation' and 'Results presentation' columns to the left. **Medium confidence:** *Thyroid* *Liver* *Female reproductive* *Developmental*
{Watanabe, 2010, 1927692@@author-year}				
Female BALB/c mice obtained from	Investigated **immune system** effects in a 28-d study	Methodology acceptable and adequately	Thorough presentation of	Design, conduct and reporting of the study

Test animal	Experimental design and exposure information	Endpoint evaluation	Results presentation	Conclusion
Kyudo Animal Laboratory, Japan	Test article purity, stability and isomeric composition was not reported Dietary Confirmation of the doses was not reported Included concurrent control Received soy-free diet and water ad libitum Design and exposure determined to be suitable for investigating all endpoints planned for in the study	described for all endpoints.	quantitative data, experimental unit, and sample size in text/figures/tables for all endpoints.	was determined to be suitable for investigating immune system effects following a 28-day exposure. **High confidence:** *Immune system*

BMD = benchmark dose; DMSO = dimethylsulfoxide; FOB = functional observational battery; GD = gestation day; GLP = good laboratory practices; PND = postnatal day; PNW = postnatal week; SD = standard deviation; SE = standard error

ED_005297A_00166423-00035

APPENDIX C. INFORMATION IN SUPPORT OF HAZARD IDENTIFICATION

C.1 TOXICOKINETICS

C.1.1 Absorption

Absorption in the human gastrointestinal (GI) tract is expected given the detection of hexabromocyclododecane (HBCD) in samples of human milk, maternal blood/cord blood, or fetal tissue, and in food samples collected in several regions of the world {NICNAS, 2012, 1443965;Environment Canada, 2011, 1937209;Rawn, 2014, 2343738;for reviews`, see \Rawn, 2014, 2238553}.

HBCD isomers were rapidly and extensively absorbed in the GI tracts of mice given single oral doses of γ-[^{14}C]-HBCD {Szabo, 2010, 787724}, α-[^{14}C]-HBCD {Szabo, 2011, 787725}, or β-HBCD {Sanders, 2013, 1927548} and rats given single oral doses of [^{14}C]- γ-HBCD (mixed with technical-grade HBCD containing ~75% γ-HBCD) {Yu, 1980, 787744}. For example, the rat study indicated nearly complete absorption; after 72 hours, 72% of the administered radioactivity was detected in feces (as nonidentified metabolites), 16% in urine, and 17% in tissues excluding the GI tract {Yu, 1980, 787744}. In studies of mice, absorption percentages between 85 and 90% were reported, based on tissue levels and cumulative fecal and urinary excretion of radioactivity {Sanders, 2013, 1927548;Szabo, 2011, 787725;Szabo, 2010, 787724}.

C.1.2 Distribution

Numerous studies of HBCD concentrations in samples of human milk, blood, fatty tissues, or fetal tissues have noted that α-HBCD is the predominant isomer detected, even though γ-HBCD is the predominant isomer in commercial HBCD products {NICNAS, 2012, 1443965;Environment Canada, 2011, 1937209;Rawn, 2014, 2343738;for reviews`, see \Rawn, 2014, 2238553}. These results indicate preferential tissue accumulation (especially in fat) of α-HBCD, compared with γ-HBCD or β-HBCD. In these studies, measurements of HBCD in maternal serum and umbilical cord serum of pregnant women have demonstrated that HBCD can cross the placenta and enter the fetal circulatory system.

In rats and mice, radioactivity from oral or intravenous (i.v.) administered [^{14}C]-HBCD distributes widely in the body, with the highest levels in fat, liver, skeletal muscle, and skin {Szabo, 2011, 787726;Yu, 1980, 787744;Sanders, 2013, 1927548;Szabo, 2010, 787724}. For example, 8 hours after administration of a single oral dose of [^{14}C]- γ-HBCD (mixed with technical-grade HBCD) in female rats, radioactivity was detected in the fat (20% of administered dose), muscle

ED_005297A_00166423-00036

(14%), and liver (7%) with smaller amounts (<1%) in the blood, heart, lung, gonads, uterus, spleen, kidney, and brain {Yu, 1980, 787744}. A similar relative distribution pattern was observed in male rats, except that the levels of radioactivity (expressed as a percentage of administered dose) in fat and muscle of males were lower (about one-half to three-quarters of the levels in females). Radioactivity in most tissues decreased over the course of 72 hours, but remained elevated in the fat. Nonpolar metabolites of HBCD accounted for all of the radioactivity in fat; isomeric composition in the fat was not determined.

The three HBCD isomers exhibit differential accumulation in mice exposed by gavage {Sanders, 2013, 1927548;Szabo, 2011, 787726;Szabo, 2010, 787724}. At 1–3 hours after single radiolabeled doses of 3 mg/kg of each isomer were given, concentrations of HBCD-derived radioactivity were highest in the liver, followed by the adrenals, kidneys, and bladder (after exposure to γ-HBCD); fat, kidneys, and lung (after exposure to β-HBCD); or blood, kidney, and brain (after exposure to α-HBCD). Tissue concentrations were markedly higher after exposure to α-HBCD (e.g., peak of 47,628 ng/g liver) than after exposure to the other isomers (peaks of 4,462 ng/g liver for β-HBCD and 2,309 ng/g liver for γ-HBCD). Tissue concentrations peaked 3–8 hours after exposure to either β- or γ-HBCD, and declined steadily thereafter. In contrast, after exposure to α-HBCD, concentrations in the skin, muscle, and adipose tissue peaked 1–2 days later, indicating redistribution and accumulation of radioactivity in these tissues. Four days after exposure to each isomer, concentrations were markedly decreased in all tissues; at that time, the highest tissue concentrations were in the fat after exposure to β- and α-HBCD (13,320 and 498 ng/g, respectively), and in the adrenal glands after exposure to γ-HBCD (492 ng/g) {Sanders, 2013, 1927548;Szabo, 2011, 787726;Szabo, 2010, 787724}. The results indicate greater deposition of α-HBCD or its metabolites in most tissues, especially fat, compared with γ-HBCD and β-HBCD. Similar findings were reported by {WIL Research, 2001, 787787@@author-year} based on data from fat tissue samples collected from rats exposed to technical-grade HBCD for 90 days at a gavage dose of 1,000 mg/kg-day; β- and γ-HBCD tissue concentrations were only 8–18% of the concentration of α-HBCD.

Sex-dependent differences in distribution were observed in rats exposed by gavage for 28 days to commercial HBCD at doses from 0.3 to 200 mg/kg-day {van der Ven, 2006, 787745}. Concentrations of total HBCD were higher (on average 5-fold higher) in livers of female than male rats over the entire dose range. Fat tissue from female rats contained HBCD concentrations approximately 4.5-fold higher than those measured in male fat tissue (based on data from two rats/sex in the 10 mg/kg-day dose group). Findings from the 90-day rat study by {WIL Research, 2001, 787787@@author-year} showed a smaller sex-dependent difference in fat tissue concentrations. In rats exposed by gavage at a dose of 1,000 mg/kg-day, the mean α-HBCD concentrations in fat tissues was only 40% greater in female rats than males at exposure day 89; the mean concentrations of β- and γ-HBCD in fat tissues in males and females were similar. Based

ED_005297A_00166423-00037

on same collections on days 2, 6, 13, 20, 27, 55, 89, 104, and 118 of the study, the patterns of distribution into fat tissues in males and females were similar.

C.1.3 Metabolism

Studies in laboratory animals and in vitro studies show that HBCD isomers can undergo stereoisomerization, hydroxylation, and debromination, and that γ-HBCD and β-HBCD are more rapidly and extensively metabolized than α-HBCD. The results also indicate that cytochrome P450 (CYP450) enzymes are involved in metabolism of HBCD, but the predominant metabolic pathways and terminal excretory metabolites have not been fully characterized. Debrominated metabolites of HBCD have been detected in human breast milk samples, suggesting that debromination steps inferred from metabolites identified in laboratory animals are applicable to humans {Abdallah, 2011, 787631}.

In vivo stereoisomerization of the γ- to the α-isomer has been demonstrated in toxicity studies of rats, and available data suggest that stereoisomerization is more important at higher doses. Dose-dependent stereoisomerization was observed in rats repeatedly exposed to commercial HBCD (with composition 10% α, 9% β, and 81% γ) by gavage {van der Ven, 2006, 787745;WIL Research, 2001, 787787} or dietary administration {van der Ven, 2009, 589273}. In these studies, the ratios of the lipid-normalized concentrations of γ-isomer to the α-isomer (measured as parent compound using liquid chromatography/mass spectrometry [LC/MS]) in liver differed from the ratios in the administered material, and these ratios declined with increasing dose. For example, in adult rats exposed for 28 days {van der Ven, 2006, 787745}, the ratios of the γ-isomer to the α-isomer (β-HBCD comprised <1.5% of the total HBCD in tissues) in females ranged from 4.2 at the low dose (0.3 mg/kg-day) to 0.4 at the high dose (200 mg/kg-day); in males, at the same doses, the ratios ranged from 2.3 at the low dose to 0.9 at the high dose. These values were all lower than the ratio of 8.1 in the administered material. This dose-dependent shift in the ratio of γ:α isomers was also observed in 11-week-old offspring of rats exposed before and during mating and during gestation and lactation {van der Ven, 2009, 589273}.

Analysis of excreta and tissues following oral administration of [^{14}C]-HBCD to rats {Yu, 1980, 787744} showed extensive metabolism of γ-HBCD. None of the radioactivity recovered in urine or feces could be identified as parent γ-HBCD following oral administration of [^{14}C]-γ-HBCD (mixed with technical-grade HBCD containing ~75% γ-HBCD). Several polar metabolites of uncharacterized structure were found in extracts of feces and urine; these metabolites constituted 88% of the cumulative radioactivity excreted during the 72 hours after dosing {Yu, 1980, 787744}.

Results of oral exposure studies in mice given the same dose of each isomer demonstrated more extensive metabolism of β- and γ-HBCD compared with α-HBCD {Sanders, 2013, 1927548;Szabo, 2011, 787725;Szabo, 2010, 787724}. For example, more radioactivity was excreted in the urine after oral dosing with β-HBCD (~45% of administered dose over 4 days) than after the same dose of either α- or γ-HBCD (~20–28% of administered dose). The urine contained only metabolites; none of the radioactivity in the urine was associated with the parent isomers

ED_005297A_00166423-00038

{Sanders, 2013, 1927548;Szabo, 2011, 787725;Szabo, 2010, 787724}. Extraction of feces samples for thin layer chromatography analysis of radioactivity showed that a significant proportion of fecal radioactivity was not extractable after exposure to α-HBCD (64%) or γ-HBCD (52%), while a lower proportion was not extractable after exposure to β-HBCD (30%). {Szabo, 2010, 787724@@author-year} hypothesized that nonextractable radioactivity in feces represented remnants from reactive metabolites covalently bound to proteins or lipids. Of the extractable radioactivity in feces, polar metabolites comprised the largest percentage of extractable fecal radioactivity after dosing with γ-HBCD (85%); polar metabolites comprised smaller percentages after dosing with α-HBCD (66%) or β-HBCD (39%). After exposure to β- and γ-HBCD, but not α-HBCD, isomerization products were detected in feces. Total extractable fecal radioactivity contained 4% β-HBCD and 7% α-HBCD after exposure to γ-HBCD, and 16% γ-HBCD after exposure to β-HBCD. No isomerization of α-HBCD was evident in any of the matrices examined. Data on the excretion of parent compound provide the strongest evidence for greater metabolism of β- and γ-HBCD compared with α-HBCD: a larger percentage of extractable fecal radioactivity was associated with parent compound after administration of α-HBCD (34%) than after dosing with β-HBCD (14%) or γ-HBCD (4%). Given that oral absorption of all three isomers was similar (85–90%), the differences in excreted parent compound appear to reflect greater metabolism of the β- and γ-isomers.

More rapid metabolism of β- and γ-HBCD relative to α-HBCD was demonstrated in in vitro studies using rat liver microsomes {Abdallah, 2014, 2343714;Zegers, 2005, 787753;Esslinger, 2011, 1927639}. Following incubation of the microsomes with NADPH and a 1:1:1 mixture of α-, β-, and γ-HBCD, LC/MS peaks for β- and γ-HBCD in the incubation fluid were greatly diminished after 90 minutes, whereas the peak for α-HBCD was essentially unchanged. In addition, degradation rates for enantiomeric isomers (+) α- and (−) α-HBCD were faster in rat liver microsomes than rates for (+) β-, (−) β-, or (−) γ-HBCD {Esslinger, 2011, 1927639}. {Abdallah, 2014, 2343714@@author-year} calculated half-times of 17.14, 11.92, and 6.34 seconds for in vitro rat liver microsomal metabolism of α-, γ-, and β-HBCD, respectively.

Hydroxylation and debromination have been identified as metabolic pathways for HBCD isomers based on partial characterization of metabolites in animal and in vitro studies. Analysis of adipose, liver, muscle, and lung tissue extracts from rats exposed to 100 mg/kg-day commercial HBCD (enriched in the γ-isomer) for 28 days identified mono- and dihydroxylated metabolites of HBCD as well as monohydroxylated derivatives of the debrominated metabolites pentabromocyclo-dodecene and tetrabromocyclododecene {Brandsma, 2009, 787646}. No sex dependent differences in metabolite profiles were observed {Brandsma, 2009, 787646}. Hydroxylated metabolites of β- and γ-HBCD, along with other unidentified metabolites, were also detected by LC/MS of incubation fluid after rat liver microsomes were incubated with a mixture of α-, β-, and γ-HBCD (1:1:1) and NADPH {Zegers, 2005, 787753}.

Although specific enzymatic pathways for metabolism of HBCD have not yet been identified, results of animal in vivo and in vitro studies are consistent with hydroxylation catalyzed by CYP450 enzymes, as suggested by the observation that HBCD induced messenger ribonucleic acid (mRNA) levels for CYP2B1/2 and CYP3A1/3 in livers of rats following 28 days of dietary exposure to commercial HBCD {Cantón, 2008, 787647;Germer, 2006, 787665}. There are no data describing the potential contribution of gut-mediated HBCD metabolism. However, it is likely that fecal metabolites are predominantly liver-derived, as only radioactive metabolites (no parent compounds) were found in the bile of mice orally exposed to α- or γ-[^{14}C]-HBCD {Szabo, 2011, 787725;Szabo, 2010, 787724}.

The available data are consistent with the proposed generalized metabolic pathways shown in Figure C-1, in which debromination occurs via undetermined enzymes and hydroxylation occurs via CYP450 oxygenases {Brandsma, 2009, 787646}. The generalized metabolic scheme in Figure C-1 does not capture in vivo and in vitro evidence that isomer-specific metabolic pathways may exist in laboratory animals, or the evidence that HBCD metabolites may be conjugated prior to excretion. {Hakk, 2012, 1927570@@author-year} found evidence for different metabolic products of γ-HBCD and α-HBCD using LC/MS analysis of extractable and nonextractable HBCD metabolites in blood, fat, brain, bile, urine, and feces collected in the toxicokinetic studies of mice exposed to radiolabeled γ-HBCD {Szabo, 2010, 787724} and α-HBCD {Szabo, 2011, 787725}. After α-HBCD exposure, two glutathione conjugates of a tri- or tetra-brominated, unsaturated C6 hydrocarbon were identified in urine, and a monohydroxylated, hexabrominated metabolite was identified in feces {Hakk, 2012, 1927570}. After γ-HBCD exposure, greater numbers of metabolites were identified in urine and feces: (1) two carboxylic acid derivatives (indicative of ring opening), a hydroxylated, pentabrominated derivative, and a putative methyl mercapturate of a tetrabrominated derivative in urine; and (2) three debrominated and oxidized derivatives in feces {Hakk, 2012, 1927570}. In rat liver microsomes tested in vitro, varied monohydroxylated HBCD products for each of several tested enantiomeric substrates were detected: one from (+) α-HBCD; three from (−) α-HBCD; two from (+) γ-HBCD; and three from (−) γ-HBCD {Esslinger, 2011, 1927639}.

[EMBED ACD.ChemSketch.20]

HBCD = hexabromocyclododecane; PBCDe = pentabromocyclododecene; TBCDe = tetrabromocyclododecene

Source: Adapted from {Brandsma, 2009, 787646@@author-year}.

Figure C-1. Proposed pathways for metabolism of HBCD in rats.

C.1.4 Elimination

Elimination of radioactivity associated with administration of HBCD isomers is rapid, with most eliminated over the first 24 hours post administration, after either oral or i.v. dosing in female mice {Sanders, 2013, 1927548;Szabo, 2011, 787725;Szabo, 2010, 787724} or oral administration in the rat {Yu, 1980, 787744}. Fecal and urinary excretion are the primary excretory pathways for absorbed HBCD, although the detection of HBCD isomers in many studies of human breast milk samples indicates that breast milk fat represents an additional elimination pathway.

The fecal:urine excretion ratios (based on samples collected over 48 hours postdosing) for absorbed HBCD in mice exposed by gavage to 3 mg/kg were approximately 2.4 for α-[^{14}C]-HBCD, 1.2 for β-[^{14}C]-HBCD, and 2.1 for γ-[^{14}C]-HBCD {Sanders, 2013, 1927548;Szabo, 2011, 787725;Szabo, 2010, 787724}. Similar ratios were seen after i.v. dosing at the same exposure level {Sanders, 2013, 1927548;Szabo, 2011, 787725;Szabo, 2010, 787724}. Together, urinary and fecal excretion 48 hours after dosing accounted for ~70% of the administered radioactivity (at 3 mg/kg) after exposure to the α isomer and ~90% after exposure to the β- and γ- isomers {Sanders, 2013, 1927548;Szabo, 2011, 787725;Szabo, 2010, 787724}. Excretion was essentially complete within 48 hours after either oral or i.v. dosing; studies evaluating elimination over longer time periods showed little additional excretion after 48 hours {Szabo, 2011, 787725;Szabo, 2010, 787724}.

The overall kinetics of urinary and fecal elimination in the rat is similar to mice, but sex-dependent differences were suggested by data in rats. Forty-eight hours after dosing with [^{14}C]-γ-HBCD (mixed with technical-grade HBCD containing ~75% γ-HBCD), fecal elimination accounted for 63% of radioactivity in four female rats and 95% in two male rats {Yu, 1980, 787744}. Over the same time frame, urinary elimination accounted for 4.8 and 15.3% of radioactivity in female and male rats, respectively.

In female mice administered α-[^{14}C]-HBCD by gavage, a dose-dependent shift in fecal elimination was observed {Szabo, 2011, 787725}. Fecal elimination accounted for about 48% of the administered radiolabel at 3 mg/kg, but only about 32% following a 100 mg/kg dose {Szabo, 2011, 787725}. The mechanism for the dose-dependent decrease in fecal excretion has not been identified; however, since radioactivity derived from absorbed α-[^{14}C]-HBCD is extensively excreted into feces, this outcome suggests a possible capacity limitation in the secretion (e.g., biliary) mechanism. This dose-dependency was not observed in similar studies of γ-[^{14}C]-HBCD in mice {Szabo, 2010, 787724}. In mice given single doses of β-[^{14}C]-HBCD of 3, 30, or 100 mg/kg, the amount of administered radioactivity in 24-hour feces was greater after 3 mg/kg (~50%) than after 100 mg/kg (~30%), but no dose-dependent difference was noted in cumulative 96-hour feces {Sanders, 2013, 1927548}.

Biphasic elimination kinetics of radioactivity from blood and tissues of mice were observed following oral administration of α-, β-, or γ-[^{14}C]-HBCD in corn oil vehicle {Sanders, 2013, 1927548;Szabo, 2011, 787725;Szabo, 2010, 787724}. Tissue half-life values for the rapid phase in mice ranged from 0.1 to 0.4 days for α-HBCD, from 0.02 to 0.2 days for β-HBCD, and from 0.3 to 1 day for γ-HBCD. Terminal tissue half-life values were longer for α-HBCD (range, 0.5–17 days) than for γ-HBCD (range, 0.8–5.2 days) or β-HBCD (0.2–7 days). In particular, the terminal half-lives for fat tissue were 17 days for α-HBCD, 3.6 days for γ-HBCD, and 2.5 days for β-HBCD, indicating that, with repeated oral exposures, α-HBCD would be expected to accumulate in fat to a greater extent than γ-HBCD or β-HBCD. Similar biphasic excretory kinetics were observed in rats following single

ED_005297A_00166423-00041

gavage doses of commercial HBCD with γ-[^{14}C]-HBCD {Yu, 1980, 787744}. Tissue excretory kinetic data for humans are not available.

Breast milk lipid represents an additional elimination pathway for HBCD, and concentrations of HBCD in human breast milk samples have been well studied; only a few reports are summarized here. Most biomonitoring studies report total HBCD concentrations in breast milk around 1 ng/g. For example, the following lipid-normalized median concentrations were reported: 0.9 ng/g lipid (range: 0.3–2.2 ng/g) and 0.4 ng/g (range: 0.2–1.2 ng/g) for populations in the United States (Texas) in 2002 and 2004, respectively {Ryan, 2014, 2343679}; 0.7 ng/g (range: 0.1–28.2 ng/g) in Ontario, Canada; 3.83 ng/g (range 1–22 ng/g) in the United Kingdom {Abdallah, 2011, 787631}; 0.6 ng/g (range: 0.6–5.7 ng/g) in Belgium {Roosens, 2010, 1927679}; and 0.86 ng/g (range: less than the limit of quantitation [LOQ] –31 ng/g) in Norway {Thomsen, 2010, 1927695}. {Ryan, 2006, 3445832@@author-year} reported that most of the HBCD detected in breast milk from Texas women was the α-isomer, whereas in Japanese women, mean lipid-normalized concentrations of α-, β-, and γ-HBCD in breast milk were 1.5, <0.1, and 2.6 ng/g, respectively {Kakimoto, 2008, 787682}.

C.1.5 Description of Toxicokinetic Models

No physiologically based pharmacokinetic (PBPK) models are available for HBCD. An unpublished, empirical two-compartment open kinetic model for orally-administered ^{14}C-HBCD was developed from data collected using Sprague-Dawley rats given single oral doses of commercial HBCD labeled with γ-[^{14}C]-HBCD (7–9 mg/kg) {Yu, 1980, 787744}. The model did not explicitly describe the metabolism of HBCD; however, the model did estimate an elimination constant. The elimination constant accounted for metabolism of HBCD and excretion of metabolites into urine and feces. The central compartment of the model comprised blood, muscle, liver, kidney, heart, spleen, lung, gonads, and uterus, and the remaining compartment represented fatty tissues. The calculated concentrations of radioactivity in the central and fat compartments were compared with respective observed concentrations in the blood and fat. The pattern of predicted values of radiolabel in blood and fat generally reflected the pattern of observed values in blood and fat. This kinetic model addressed the distribution of radioactivity only, and did not explicitly describe metabolism.

{Aylward, 2011, 1927641@@author-year} proposed the use of lipid-adjusted tissue concentrations of HBCD as an internal dose metric that would reduce uncertainties associated with the inter- and intraspecies extrapolation based on external dose. They derived a simple first-order elimination model to estimate the steady-state lipid concentration of HBCD (in ng/g lipid) corresponding to a given daily HBCD intake (in mg/kg-day) as follows:

$$D = C_l \times F_l \times k$$

where D = chronic daily dose in mg/kg day, C_l = lipid concentration (in mg/kg lipid), F_l = fraction of body weight that is lipid (assumed to be 25%), and k = elimination rate calculated from the half-life (HL, assumed to be 64 days in days) as k = ln (2)/HL.

As noted by {Aylward, 2011, 1927641@@author-year}, uncertainty in the steady-state lipid concentration of HBCD derived using this model comes from the assumed values for the half-life of HBCD and the proportion of lipid in the body. If used for purposes of interspecies extrapolation, uncertainty is also introduced by potential toxicokinetics differences across species (e.g., differences in rates of metabolism of the different HBCD isomers), and consideration of whether summed or isomer-specific doses should be used. If humans clear individual isomers at a different rate than animals, and if the toxicity of individual isomers differs, the internal summed dose could either over- or underpredict the response. Finally, it should be noted that a systematic examination of whether lipid-adjusted tissue concentrations better correlate with response than other measures of dose (e.g., blood concentration, total concentration) has not been conducted.

C.2 ~~SUMMARY OF GENOTOXICITY~~ OTHER TOXICITY INFORMATION

Commented [RS5]: Because the evidence for male reproductive and immune system effects were deterimined to be inadequate, these sections were moved from the Toxicological Review to the Supplemental Inforamtion volume in order to focus the Toxicological Review on those hazards with sufficient evidence to support hazard conclusions.

C.2.1 Male Reproductive Effects

Human Evidence

Epidemiological studies evaluating HBCD exposure and reproductive endpoints include a birth cohort {Meijer, 2012, 1401499} and a cross-sectional study of male infertility patients {Johnson, 2013, 1676758} (Table C-11-5). The birth cohort study in the Netherlands examined maternal serum HBCD levels in relation to male infants' testes volume and penile length at 3 and 18 months (n = 44) as well as steroidal and gonadotropin hormone levels at 3 months (n = 34) {Meijer, 2012, 1401499}. Effect estimates for the association with testes volume or penile length were not provided, but were reported to be not statistically significant. A weak to moderate correlation coefficient (r = −0.31; 0.05 < p < 0.10) was observed between maternal serum HBCD and free testosterone. No other effects on steroidal or gonadotropin hormones were associated with serum HBCD levels (effect estimates not provided). A study examining the relationship between HBCD concentrations in household dust and reproductive hormones in 38 adult men from the United States attending an infertility clinic {Johnson, 2013, 1676758} reported statistically significant correlations for decreased sex hormone binding globulin (SHBG) (r = −0.35; p = 0.03) and increased free androgen index (testosterone/SHBG) (r = 0.46; p = 0.004); the effect on the free androgen index was likely due to decreased SHBG levels, as testosterone concentrations did not appear to be related to HBCD exposure. Correlation coefficients for other hormones were not reported, but were described as not statistically significant {Johnson, 2013, 1676758}.

Overall, given the limited evidence for male reproductive effects associated with HBCD exposure and the low confidence in the two studies that evaluated male reproductive outcomes (see Table C-11-5), the database was considered inadequate to draw conclusions regarding the relationship between HBCD exposure and male reproductive effects in humans.

Animal Evidence

Evidence to inform the potential for HBCD to induce male reproductive effects, including reproductive differentiation and development, spermatogenic measures, and reproductive organ weights, comes from five studies in rats {Ema, 2008, 787657;Saegusa, 2009, 787721;van der Ven, 2009, 589273;WIL Research, 2001, 787787;van der Ven, 2006, 787745} with exposure durations ranging from 28 days to two generations. Evidence pertaining to male reproductive effects in experimental animals following oral exposure to HBCD is summarized in Table C-21-7 and Figure C-21-6. Effect categories with stronger evidence are presented first, with individual studies ordered by study duration and then species. If not otherwise indicated, endpoint measurements were made in adults.

The available evidence for an association between HBCD exposure and male reproductive effects in experimental animals is inconclusive (Table C-11-7). One study found a significant dose-related increase in AGD, a measure of reproductive differentiation and development, only on PND 4 {van der Ven, 2009, 589273} and the biological significance of increased AGD is unclear. {van der Ven, 2009, 589273@@author-year} also reported a significant trend with dose for epididymal sperm with separate heads in rats continuously exposed to HBCD from gestation through PNW 11, but not after a 28-day exposure in adults {van der Ven, 2006, 787745}. Statistically significant increases (9–12% relative to control) in relative testis weight were reported for PND 26 F1 rats in all three dose groups (approximately 17–1,500 mg/kg-day) in a two-generation reproductive study {Ema, 2008, 787657}, but not in 15-week F1 males or PND 26 F2 males in the same study. Relative testes weights in HBCD-exposed rats were increased (6–7%) in {WIL Research, 2001, 787787@@author-year} and decreased (4–7%) in {Saegusa, 2009, 787721@@author-year}; in both studies, changes were not statistically significantly different. Two studies reported statistically significant changes in relative prostate weight in high-dose animals; however, the direction of the effect was not consistent across studies, with {Ema, 2008, 787657@@author-year} reporting a decrease and {WIL Research, 2001, 787787@@author-year} reporting an increase. Furthermore, this effect was no longer present following a 4-week recovery period {WIL Research, 2001, 787787}. No other dose-related effects were observed for other measures of male reproductive differentiation and development {Ema, 2008, 787657;van der Ven, 2009, 589273;Saegusa, 2009, 787721}, spermatogenic measures {Ema, 2008, 787657;van der Ven, 2006, 787745;van der Ven, 2009, 589273;WIL Research, 2001, 787787}, or male reproductive organ weights {Ema, 2008, 787657;van der Ven, 2009, 589273;Saegusa, 2009, 787721;WIL Research, 2001, 787787}.

Table C-11-5. Evidence pertaining to male reproductive toxicity of HBCD in humans

Reference and study design	Results
{Meijer, 2012, 1401499@@author-year} (the Netherlands, COMPARE cohort, 2001–2002) **Population:** Birth cohort, 90 singleton, term births, 55 healthy boys, assessed at 3 mo (n = 55) and 18 mo (n = 52); 44 with HBCD measures, 45 with hormone measures, 34 with both measures **Exposure measures:** Prenatal exposure, maternal serum at 35th week of pregnancy 1,2,5,6,9,10-HBCD (HBCD) detected in 43 of 44 samples LOD 0.8 pg/g serum; LOQ = 9 pg/g serum Median 0.7 (range: <LOD–7.4) ng/g lipid **Effect measures:** Reproductive hormones (serum, collected at 3 mo) {immunoassay details in \Laven, 2004, 2238548} • testosterone • SHBG • FSH • LH • estradiol • inhibin B Testes volume, measured by ultrasound (ages 3 and 18 mo); penile length (ages 3 and 18 mo) **Analysis:** Spearman correlation **Study evaluation[a]:** [EMBED PBrush] Limited analysis and inadequate reporting of results; small sample size	Spearman correlation between HBCD in maternal serum and free testosterone: r = −0.31 (0.05 < *p*-value < 0.10). Correlations with other hormones noted as not statistically significant, but effect estimates were not reported. No significant correlations between prenatal exposure to HBCD and testes volume or penile length were found (data not shown).
{Johnson, 2013, 1676758@@author-year} (USA, 2002–2003) **Population:** 38 men (18–54 yrs old), from couples seeking infertility treatment; approximately 65% participation into general study; participation rate in the vacuum bag collection phase not reported **Exposure measures:** HBCD exposure from vacuum bag dust; three main stereoisomers of HBCD presented together; HBCD detected in 97% of samples; LOD not reported; median 246 ng/g dust (90th percentile 1,103 ng/g dust) **Effect measures:** Non-fasting blood sample {immunoassay details in \Meeker, 2008, 2238550} testosterone SHBG FSH LH estradiol	Spearman r (*p*-value) Free androgen index (testosterone/SHBG): 0.46 (*p* = 0.004) SHBG: −0.35[a] (*p* = 0.03) Multivariate models adjusted for age and BMI reportedly produced similar results to the bivariate results (data not reported for HBCD). Results for other hormones not shown. Note that HBCD was not strongly correlated with other flame retardants measured (Spearman correlation coefficients ranging from −0.20 to 0.27, all *p*-values > 0.10)

ED_005297A_00166423-00045

Reference and study design	Results
inhibin B prolactin **Analysis:** All variables analyzed as continuous variables; Spearman's correlation between HBCD in house dust and serum hormone levels; multivariable models adjusted for age and BMI, but results for HBCD model results not reported **Study evaluation[a]:** [EMBED PBrush] Limited analysis and inadequate reporting of results; small sample size	

[a]Evaluation of sources of bias or study limitations (see Systematic Review Methods/Epidemiology Studies, and Appendix B, Table B-3): P = population selection; E = exposure misclassification; O = outcome misclassification; C = confounding; A = analysis; Oth = other feature affecting interpretation of results. Extent of column shading reflects degree of limitation.

Table C-21-7. Evidence pertaining to male reproductive effects in animals following exposure to HBCD

Reference and study design	Results				
Reproductive differentiation and development					
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation	**Doses** (mg/kg-d)				
	F1 offspring[a]	**0**	**17**	**168**	**1,570**
	F2 offspring[a]	**0**	**15**	**139**	**1,360**
	AGD (mm)				
	Male, F1, PND 4 (n = 18–24 litters)				
	Mean (SD)	5.37 (0.41)	5.44 (0.36)	5.38 (0.32)	5.20 (0.51)
	% change[b]	–	1%	0%	−3%
	Male, F2, PND 4 (n = 19–22 litters)				
	Mean (SD)	5.12 (0.54)	5.12 (0.41)	5.04 (0.42)	4.84 (0.39)
	% change[b]	–	0%	−2%	−5%

Reference and study design	Results								
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F0: exposure started one spermatogenic	**Doses** (mg/kg-d)								
		0	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	AGD (mm)								
	Male, F1, PND 4 (n ≥ 14)[c] **								
	Mean (SD)	4.6 (0.8)	5.1 (1.1)	4.7 (0.8)	4.8 (1.0)	5.0 (0.8)	5.0 (0.9)	4.5 (0.8)	5.4 (1.0)
	% change[b]	–	11%	2%	4%	9%	9%	−2%	17%
	Male, F1, PND 7 (n ≥ 14)[c]								

ED_005297A_00166423-00046

Reference and study design	Results								
cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/ lactation; dietary exposure post weaning through PNW 11	Mean (SD)	6.2 (1.2)	6.7 (1.2)	5.5 (1.1)	6.4 (1.4)	6.1 (1.3)	6.0 (1.3)	6.6 (1.0)	6.3 (1.2)
	% change[b]	–	8%	−11%	3%	−2%	−3%	6%	2%
	Male, F1, PND 21 (n ≥ 14)[c]								
	Mean (SD)	19.0 (6.0)	19.1 (4.1)	14.8 (2.6)		18.7 (2.9)	18.3 (5.5)	18.9 (6.1)	16.0 (2.2)
	% change[b]	–	1%	−22%	n/a	−2%	−4%	−1%	−16%
	Value for male F1 PND 21 rats at 1 mg/kg-d was "n/a" in study report.								
{Saegusa, 2009, 787721@@author-year} Rats, Crj:CD(SD)IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11	Doses (mg/kg-d)[d]	**0**	**15**	**146**	**1,505**				
	AGD (mm)								
	Male, F1, PND 1 (n = 10 litters)								
	Mean (SD)	3.88 (0.23)	3.96 (0.20)	4.08 (0.30)	4.01 (0.23)				
	% change[b]	–	2%	5%	3%				
Spermatogenic measures									
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/ lactation; dietary exposure post weaning through PNW 11	Doses (mg/kg-d)	**0**	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	Epididymal sperm with separate heads (% of total)								
	Male, F1, PNW 11 (n = 4–5)**								
	Mean (SD)	4.2 (1.7)	3.8 (2.9)	7.5 (8.1)	2.2 (1.9)	4.4 (1.9)	4.1 (2.1)	5.0 (1.8)	0.8 (0.8)
	% change[b]	–	−10%	79%	−48%	5%	−2%	19%	−81%
{van der Ven, 2006, 787745@@author-year} Rats, Wistar Gavage	Doses (mg/kg-d)	**0**	**0.3**	**1**	**3**	**10**	**30**	**100**	**200**
	Epididymal sperm with separate heads (% of total)								
	Male (n = 4–5)								
	Mean (SD)	5.3 (2.9)	3.8 (2.2)	7.4 (3.2)	4.7 (3.4)	5.1 (4.0)	6.8 (4.1)	3.5 (2.7)	5.1 (3.6)

Reference and study design	Results								
28-d exposure starting on PNW 11	% change[b]	–	−28%	40%	−11%	−4%	28%	−34%	−4%
Reproductive organ weights									
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation	**Doses** (mg/kg-d)								
	F1, offspring[a]	**0**		**17**		**168**		**1,570**	
	Male, F1, adult	**0**		**11**		**115**		**1,142**	
	F2, offspring[a]	**0**		**15**		**139**		**1,360**	
	Relative epididymis weight (left and right) (mg/100 g BW)								
	Male, F1, PND 26 (n = 17−23)								
	Mean (SD)	85.9 (9.8)		86.7 (10.3)		89.3 (7.5)		89.9 (15.3)	
	% change[b]	–		1%		4%		5%	
	Male, F1 adult (n = 22−24)								
	Mean (SD)	223 (24)		232 (24)		210 (19)		234 (23)	
	% change[b]	–		4%		−6%		5%	
	Male, F2, PND 26 (n = 13−22)								
	Mean (SD)	90.7 (14.1)		87.2 (10.6)		87.3 (9.6)		96.2 (10.5)	
	% change[b]	–		−4%		−4%		6%	
	Relative testis weight (left and right) (mg/100 g BW)								
	Male, F1, PND 26 (n = 17−23)								
	Mean (SD)	0.57 (0.07)		0.61* (0.06)		0.62* (0.06)		0.63* (0.07)	
	% change[b]	–		9%		9%		12%	
	Male, F1 adult (n = 22−24)								
	Mean (SD)	0.60 (0.07)		0.61 (0.05)		0.58 (0.06)		0.59 (0.07)	
	% change[b]	–		2%		−4%		−1%	
	Male, F2, PND 26 (n = 13−22)								
	Mean (SD)	0.57 (0.01)		0.60 (0.06)		0.57 (0.09)		0.59 (0.05)	
	% change[b]	–		5%		0%		3%	
	Relative ventral prostate weight (mg/100 g BW)								
	Male, F1, PND 26 (n = 17−23)								
	Mean (SD)	46.4 (10.3)		47.1 (8.8)		48.2 (7.3)		44.5 (11.1)	
	% change[b]	–		2%		4%		−4%	
	Male, F1 adult (n = 22−24)								
	Mean (SD)	137 (28)		135 (34)		131 (30)		135 (22)	
	% change[b]	–		−1%		−4%		−1%	
	Male, F2, PND 26 (n = 13−22)								
	Mean (SD)	50.2 (9.3)		50.2 (10.7)		50.8 (9.6)		47.3 (15.8)	
	% change[b]	–		0%		1%		−6%	
	Doses (mg/kg-d)								
	Male, F1	**0**	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**

ED_005297A_00166423-00048

Reference and study design	Results								
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/ lactation; dietary exposure post weaning through PNW 11	**Absolute epididymis weight** (left and right) (g)								
	Male, F1, PNW 11 (n = 4–5)								
	Mean (SD)	0.95 (0.13)	0.88 (0.13)	0.95 (0.12)	1.00 (0.06)	0.90 (0.09)	0.85 (0.13)	0.98 (0.14)	0.82 (0.06)
	% change[b]	–	–7%	0%	5%	–5%	–11%	3%	–14%
	Absolute testis weight (left and right) (g)								
	Male, F1, PNW 11 (n = 4–5)**								
	Mean (SD)	3.01 (0.17)	2.91 (0.08)	3.07 (0.42)	3.18 (0.20)	2.88 (0.28)	2.82 (0.07)	2.97 (0.25)	2.60 (0.06)
	% change[b]	–	–3%	2%	6%	–4%	–6%	–1%	–14%
	Absolute prostate weight (g)								
	Male, F1, PNW 11 (n = 4–5)**								
	Mean (SD)	0.66 (0.18)	0.73 (0.21)	0.57 (0.15)	0.73 (0.21)	0.57 (0.12)	0.58 (0.07)	0.67 (0.09)	0.42 (0.13)
	% change[b]	–	11%	–14%	11%	–14%	–12%	2%	–36%
	Absolute seminiferous vesicle weight (g)								
	Male, F1, PNW 11 (n = 4–5)								
	Mean (SD)	1.00 (0.40)	1.07 (0.22)	1.32 (0.23)	1.14 (0.29)	1.21 (0.09)	1.07 (0.29)	1.21 (0.25)	1.09 (0.27)
	% change[b]	–	7%	32%	14%	21%	7%	21%	9%

Reference and study design	Results				
{WIL Research, 2001, 787787@@author-year} Rats, Crl:CD(SD)IGS BR Gavage 90 d exposure starting on ~PNW 7 followed by a 28-d recovery period Recovery data not shown	**Doses** (mg/kg-d)				
	Male	**0**	**100**	**300**	**1,000**
	Relative prostate weight (g/100 g BW)				
	Male (n = 9–10)				
	Mean (SD)	0.18 (0.03)	0.19 (0.03)	0.21 (0.04)	0.26 (0.05)
	% change[b]	–	3%	17%	42%
	Relative testis weight (left) (g/100 g BW)				
	Male (n = 9–10)				
	Mean (SD)	0.30 (0.08)	0.31 (0.04)	0.31 (0.04)	0.32 (0.04)
	% change[b]	–	4%	2%	7%
	Relative testis weight (right) (g/100 g BW)				
	Male (n = 9–10)				
	Mean (SD)	0.31 (0.07)	0.31 (0.04)	0.31 (0.04)	0.32 (0.05)
	% change[b]	–	0%	1%	6%
	Relative cauda epididymis weight (left) (g/100 g BW)				

Reference and study design	Results				
	Male (n = 9–10)				
	Mean (SD)	0.05 (0.01)	0.06 (0.01)	0.06 (0.01)	0.06 (0.01)
	% change[b]	–	9%	6%	15%
	Relative cauda epididymis weight (right) (g/100 g BW)				
	Male (n = 9–10)				
	Mean (SD)	0.05 (0.01)	0.06 (0.01)	0.06 (0.01)	0.06 (0.01)
	% change[b]	–	6%	4%	17%
	Relative epididymis weight (left) (g/100 g BW)				
	Male (n = 9–10)				
	Mean (SD)	0.12 (0.02)	0.13 (0.01)	0.12 (0.02)	0.14 (0.01)
	% change[b]	–	8%	3%	13%
	Relative epididymis weight (right) (g/100 g BW)				
	Male (n = 9–10)				
	Mean (SD)	0.12 (0.04)	0.13 (0.01)	0.13 (0.01)	0.14 (0.02)
	% change[b]	–	8%	3%	16%
{Saegusa, 2009, 787721@@author-year} Rats, Crj:CD(SD)IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11	**Doses** (mg/kg-d)[d]				
	Male, F1	**0**	**14.8**	**146.3**	**1,505**
	Relative epididymis weight (left and right) (g/100 g BW)				
	Male, F1, PND 20 (n = 10)				
	Mean (SD)	0.06 (0.02)	0.07 (0.01)	0.07 (0.01)	0.07 (0.01)
	% change[b]	–	8%	13%	8%
	Male, F1 adult, PNW 11 (n = 10)				
	Mean (SD)	0.23 (0.02)	0.21* (0.01)	0.22 (0.02)	0.21 (0.01)
	% change[b]	–	–9%	–4%	–9%
	Relative testis weight (left and right) (g/100 g BW)				
	Male, F1, PND 20 (n = 10)				
	Mean (SD)	0.43 (0.04)	0.43 (0.03)	0.43 (0.05)	0.40 (0.03)
	% change[b]	–	0%	0%	–7%
	Male, F1 adult, PNW 11 (n = 10)				
	Mean (SD)	0.77 (0.07)	0.73 (0.04)	0.78 (0.09)	0.74 (0.05)
	% change[b]	–	–5%	1%	–4%
	Relative dorsolateral prostate weight (mg/100 g BW)				
	Male, F1 adult, PNW 11 (n = 10)				
	Mean (SD)	0.13 (0.03)	0.13 (0.01)	0.14 (0.03)	0.13 (0.02)
	% change[b]	–	0%	8%	0%
	Relative ventral prostate weight (mg/100 g BW)				
	Male, F1 adult, PNW 11 (n = 10)				
	Mean (SD)	0.13 (0.02)	0.13 (0.04)	0.12 (0.03)	0.12 (0.01)
	% change[b]	–	0%	–8%	–8%

Reference and study design	Results				
	Relative seminal vesicle weight (mg/100 g BW)				
	Male, F1 adult, PNW 11 (n = 10)				
	Mean (SD)	0.27 (0.05)	0.26 (0.03)	0.26 (0.05)	0.26 (0.05)
	% change[b]	–	−4%	−4%	−4%

*Statistically significantly different from the control at $p < 0.05$ as reported by study authors.
**Significant dose response trend as reported by study authors.
[a]F1 and F2 offspring doses presented as mean maternal gestational and lactational F0 and F1 doses, respectively.
[b]Percent change compared to control calculated as: (treated value − control value)/control value × 100.
[c]Exact number of animals examined per dose group was unclear in the published paper.
[d]TWAs for each exposure group were calculated by: (1) multiplying the measured HBCD intake (mg/kg-day) reported by the study authors for GDs 10−20, PND 1−9, and PND 9−20 by the number of inclusive days of exposure for each time period; (2) adding the resulting products together; and (3) dividing the sum by the total number of inclusive days (33) of HBCD exposure. Example: 100 ppm = (8.1 mg/kg-day × 11 days) + (14.3 mg/kg-day × 10 days) + (21.3 mg/kg-day × 12 days)/33 days = 14.8 mg/kg-day.

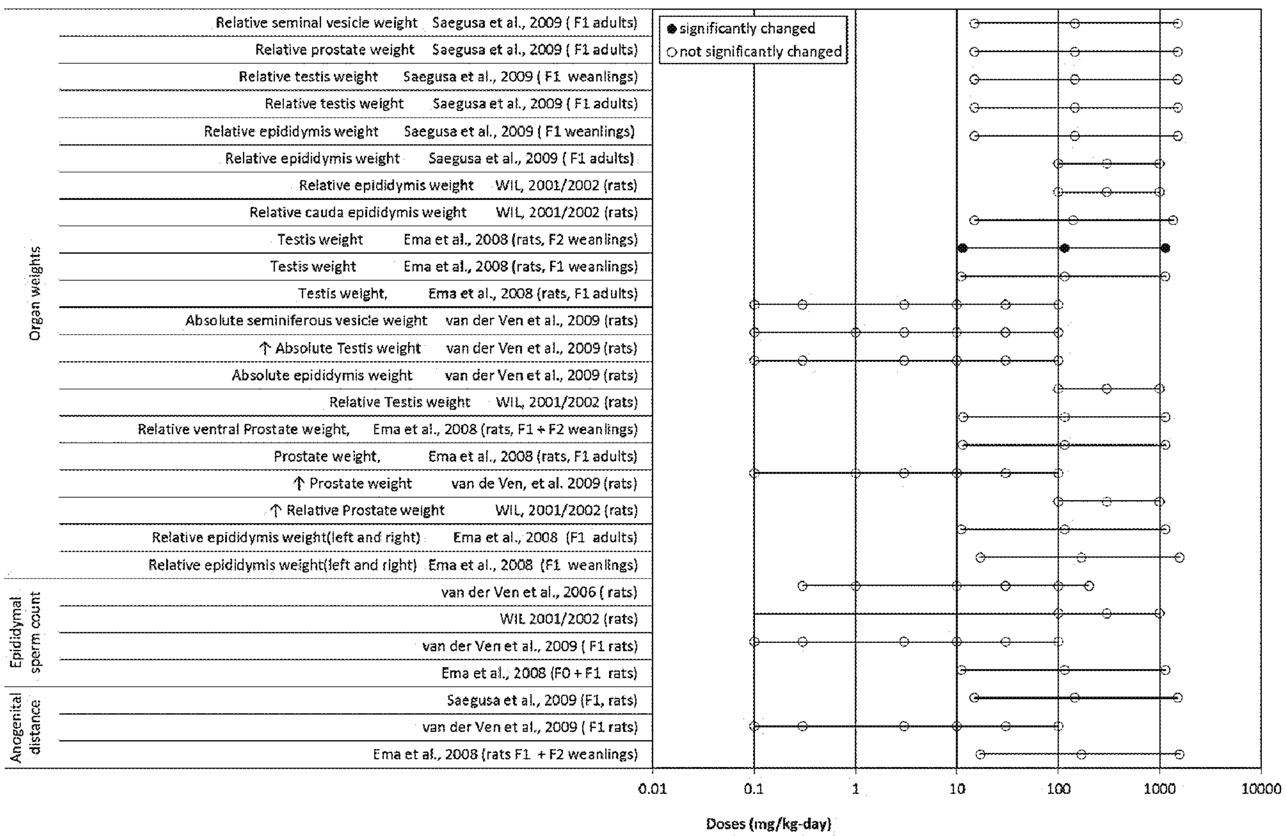


Figure C-21-6. Exposure response array of male reproductive system effects following oral exposure.

Commented [LA6]: New ER arrays are housed in HAWC.

Mechanistic Evidence

See Section 1.2.3 of the Toxicological Review (Mechanistic Evidence).

Integration of Evidence

Two epidemiological studies investigated reproductive endpoints in male subjects from a birth cohort and adult males seeking infertility treatments {Meijer, 2012, 1401499;Johnson, 2013, 1676758}; these studies, both considered to be of low confidence, provide some evidence of an association between HBCD exposure and altered serum testosterone and SHGB levels, but not other hormones. Overall, the human studies are inadequate to draw conclusions regarding the relationship between HBCD exposure and male reproductive effects.

In animal studies, no consistent effects on male reproductive organ weights, reproductive development, hormone concentrations, or spermatogenic measures were associated with 28-day, 90-day, or developmental exposure to HBCD {WIL Research, 2001, 787787;Ema, 2008, 787657;Saegusa, 2009, 787721;van der Ven, 2006, 787745;van der Ven, 2009, 589273}. There is inadequate information to assess male reproductive toxicity following exposure to HBCD (see Section 1.2.3 of the Toxicological Review, Male Reproductive Effects).

C.2.2 Immune System Effects

Human Evidence

The potential for HBCD to affect the immune system has not been investigated in humans.

Animal Evidence

The potential for HBCD to affect the immune system has been examined in eight studies in rats {van der Ven, 2009, 589273;van der Ven, 2006, 787745;Hachisuka, 2010, 2919532;Ema, 2008, 787657;WIL Research, 1997, 787758;WIL Research, 2001, 787787} and mice {Maranghi, 2013, 1927558;Watanabe, 2010, 1927692}, with exposures ranging from a 28-day exposure in adults to continuous exposure across two generations.

Discussion of immune-related effects of HBCD is organized first by age of exposure (i.e., developmental or adult) and second by the type of endpoint evaluated (i.e., functional or observational). Exposure timing is an important factor that may influence the effect of chemical exposure on immune function, particularly for early-life exposure studies. In rodents, immune development occurs in a series of discrete stages until approximately PND 42. The developing immune system is susceptible to perturbation resulting from chemical exposure, and exposures during this period may result in distinct toxicological consequences that would not be observed in animals exposed only as adults {Burns-Naas, 2008, 1011861}. With regard to the type of endpoint evaluated, functional immune outcomes, including response to challenge with an infectious agent or immunization with a foreign antigen, are the most relevant and sensitive for determining potential immunotoxicity because the primary role of the immune system is to protect host integrity from

ED_005297A_00166423-00053

foreign challenge and potential insult. Laboratory animals are housed in environments that limit their exposure to antigenic stimulation or infectious agents, and their immune systems are typically in a resting state {WHO, 2012, 1249755}. In the absence of a foreign challenge, observational endpoints, including structural alterations or changes in immune cell populations, can provide information about immune system effects, but are considered less sensitive and predictive {Luster, 2005, 2174509}.

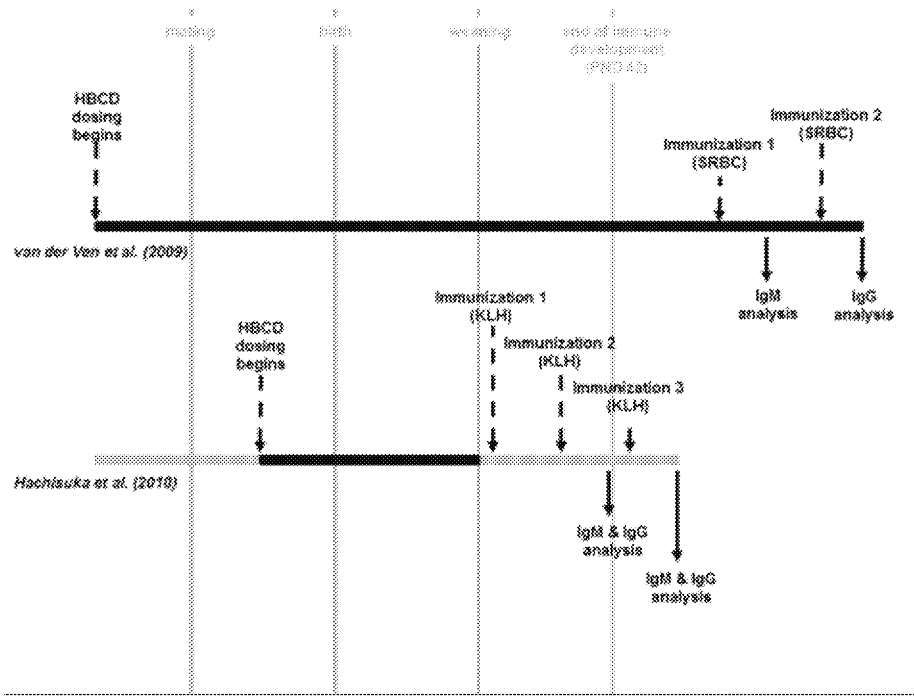
A summary of the evidence pertaining to functional and observational immune system effects in experimental animals is presented in Tables C-31-11, C-41-12, and C-51-13 and Figure C-41-10. Studies are ordered within effect categories by decreasing exposure duration and then species.

Developmental exposure

Functional immune effects

Changes in functional immune endpoints (immunoglobulin G [IgG] and immunoglobulin [IgM] antibody production in response to foreign antigens) following developmental HBCD exposures were evaluated in two one-generation reproductive toxicity studies in male {van der Ven, 2009, 589273} or female rats {Hachisuka, 2010, 2919532} (see Table C-31-11 and Figure C-41-10). Statistically significant changes in IgG levels were reported in both studies, but with opposite directions of effect; males exposed to up to 100 mg/kg-day showed a dose-dependent increase in IgG, whereas females exposed to approximately 1,500 mg/kg-day showed a decrease. Differences in the design of these two studies, including timing of exposure, immune challenge, and titer measurement (Figure C-31-9), may have contributed to the inconsistent results. IgM activity was unaffected in {van der Ven, 2009, 589273@@author-year} and results were not reported by {Hachisuka, 2010, 2919532@@author-year}. {van der Ven, 2009, 589273@@author-year} also evaluated natural killer (NK) cell activity and found no treatment-related effects.

ED_005297A_00166423-00054



KLH = keyhole limpet hemocyanin; SRBC = sheep red blood cell

Horizontal lines represent the experimental timelines, with black indicating the time period when HBCD was administered (i.e., from 2 weeks prior to mating through IgG analysis in {van der Ven, 2009, 589273@@author-year}, and from GD 10 to PND 21 in {Hachisuka, 2010, 2919532@@author-year}).

Figure C-31-9. Comparison of study designs used by {van der Ven, 2009, 589273@@author-year} and {Hachisuka, 2010, 2919532@@author-year}.

Observational immune effects

Five studies evaluated effects on observational immune parameters, including organ weights, hematology, and histopathology, in developmentally-exposed rats {Ema, 2008, 787657;van der Ven, 2009, 589273;Hachisuka, 2010, 2919532;Saegusa, 2009, 787721} or mice {Maranghi, 2013, 1927558} (see Table C-41-12 and Figure C-41-10).

Thymus weights showed significant dose-response trends in male and female adult rats (PNW 11) continuously exposed to HBCD at doses up to 100 mg/kg-day {van der Ven, 2009, 589273} and in female F2 weanlings exposed to approximately 1,300 mg/kg-day HBCD throughout gestation and lactation {Ema, 2008, 787657}. Spleen weight was reduced in both male and female F2 weanlings from the 1,300 mg/kg-day dose group {Ema, 2008, 787657}. A significant positive trend was also reported for absolute popliteal lymph node weight in PNW 11 male, but not female, rats {van der Ven, 2009, 589273}. No other treatment-related effects were reported for thymus {Hachisuka, 2010, 2919532;Saegusa, 2009, 787721;Maranghi, 2013, 1927558} or spleen weights {Hachisuka, 2010, 2919532;Saegusa, 2009, 787721;Maranghi, 2013, 1927558;van der Ven, 2009, 589273}.

Hematological analyses revealed significant treatment-related effects on several blood immune cell populations, although the pattern of effect was variable across studies, sex, and time point. Total white blood cell (WBC) count was measured in three studies. {Hachisuka, 2010, 2919532@@author-year} reported statistically significant increases in WBC count in HBCD-exposed male rats on PNWs 3 and 11 (approximately 8 weeks after the end of the exposure). In contrast, {van der Ven, 2009, 589273@@author-year} reported a significant dose-related decrease in continuously exposed PNW 11 male rats, and {Ema, 2008, 787657@@author-year} found no effect on total WBCs of F1 males or females. In addition to total WBCs, several subpopulations were measured. {van der Ven, 2009, 589273@@author-year} found a significant dose-related increase and decrease in the fraction of neutrophils and lymphocytes, respectively. The magnitude of the lymphocyte change was small (≤4% change from control) and the biological significance is unclear. {Hachisuka, 2010, 2919532@@author-year} also measured subpopulations of several leukocyte subtypes. On PNW 3, high-dose (1,505 mg/kg-day HBCD) male rats showed a decrease in activated T-cell and NK cell fractions and an increase in inactive B-cell fractions; however, cell fractions returned to control levels by PNW 11.

{Hachisuka, 2010, 2919532@@author-year} and {van der Ven, 2009, 589273@@author-year} reported inconsistent effects on splenic NK and cytotoxic T-cell populations. {Hachisuka, 2010, 2919532@@author-year} reported a statistically significant decrease in the NK cell fraction (e.g., CD4NKT cells, PNW 3) and an increase in the cytotoxic T-cell fraction in adult rats (CD8+ cells, PNW 11) that were gestationally and lactationally exposed to HBCD. In contrast, male rats continuously exposed through PNW 11 showed a dose-dependent increase in the NK cell fraction and no change in the cytotoxic T-cell fraction. No other treatment-related effects were observed for other immune cell counts in the spleen {van der Ven, 2009, 589273}.

Immune cell counts were also measured in the thymus {Hachisuka, 2010, 2919532} and bone marrow {van der Ven, 2009, 589273}. Rats showed decreases in the thymus fraction of active and regulatory T-cells and an increase in NK cells on PNW 3 and PNW 11, respectively {Hachisuka, 2010, 2919532}. WBC counts in bone marrow showed an increasing dose-related trend in adult males continuously exposed to HBCD at doses up to 100 mg/kg-day {van der Ven, 2009, 589273}.

Histological examination of immune-related tissues showed limited changes with no clear pattern of effect. Thymus tissues showed increased incidence of "starry sky" appearance {Hachisuka, 2010, 2919532} and blurring of the corticomedullary demarcation {Maranghi, 2013, 1927558} in rats and mice, respectively. In the spleen, increased incidence of marginal zone enlargement was also observed in adult (PNW 11) rats continuously exposed to 100 mg/kg-day

ED_005297A_00166423-00056

HBCD {van der Ven, 2009, 589273}. No other treatment-related histological changes were observed {Hachisuka, 2010, 2919532;van der Ven, 2009, 589273;Ema, 2008, 787657}.

Adult exposure

Functional immune effects

Two studies evaluated functional immune endpoints following adult exposure to HBCD for 28 days {van der Ven, 2006, 787745;Watanabe, 2010, 1927692}. No statistically significant changes were observed in NK cell activity in adult male rats {van der Ven, 2006, 787745} or host immunity infection in female mice {Watanabe, 2010, 1927692}.

Observational immune effects

Treatment related effects on organ weight, hematology, and histopathology were evaluated in four rat studies {van der Ven, 2006, 787745;Ema, 2008, 787657;WIL Research, 1997, 787758;WIL Research, 2001, 787787} (see Table C-51-12 and Figure C-41-10). Trends identified by the authors as statistically significant were reported for absolute thymus weight in male rats and for absolute spleen weight in female rats administered up to 200 mg/kg-day for 28 days {van der Ven, 2006, 787745}. In both cases, effects were not consistent across sexes, the magnitude of the effect was small, and the biological significance of these changes is unclear. Hematological analyses revealed a statistically significant reduction in the percentage of stabform and segmented neutrophils and increase in the lymphocyte fraction of F0 females exposed to HBCD for 14 weeks {Ema, 2008, 787657}; however, these effects were only seen in the low-dose group (approximately 14 mg/kg-day) in this study and not in a second study involving adult exposure {van der Ven, 2006, 787745}. Total splenocyte number was decreased in adult male rats in the 28-day study by {van der Ven, 2006, 787745@@author-year}. No other observational immune endpoints were affected {Ema, 2008, 787657;WIL Research, 1997, 787758;WIL Research, 2001, 787787}.

Table C-31-11. Evidence pertaining to functional immune system effects in animals following exposure to HBCD during development

Reference and study design	Results								
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11	**Doses (mg/kg-d)**								
	Male, F1	**0**	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	SRBC antibody titers IgG (extinction)								
	Male, F1, PNW 11 (n = 2–4)**								
	Mean (SD)	0.182 (0.128)	0.362 (0.333)	0.174 (0.143)	0.233 (0.169)	0.152 (0.180)	0.444 (0.143)	0.856 (0.231)	0.469 (0.205)
	% change[a]	–	99%	−4%	28%	−16%	144%	370%	158%
	Animals (males only) immunized with SRBCs on PNWs 8 and 10.								

Reference and study design	Results				
{Hachisuka, 2010, 2919532@@author-year} Rats, SD:IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk recovery period through PNW 11	**Doses** (mg/kg-d)[b]				
	Female, F1	**0**	**14.8**	**146.3**	**1,505**
	Antibody IgG responses to KLH (titer)				
	Female, F1, PND 40 (n = 7–8, estimated from graph)				
	Mean	139,452	63,196	95,592	42,548*
	% change[a]	–	−55%	−31%	−69%
	Data were digitized from figure; animals (females only) challenged with KLH on PNDs 23 and 33. IgM titers (enzyme-linked immunosorbent assay) were measured on PND 40.				

*Statistically significantly different from the control at $p < 0.05$.
**Significant dose response trend.
[a]Percent change compared to control calculated as: (treated value − control value)/control value × 100.
[b]TWAs for each exposure group were calculated by: (1) multiplying the measured HBCD intake (mg/kg-day) reported by the study authors for GDs 10–20, PNDs 1–9, and PNDs 9–20 by the number of inclusive days of exposure for each time period; (2) adding the resulting products together; and (3) dividing the sum by the total number of inclusive days (33) of HBCD exposure. Example: 100 ppm = (8.1 mg/kg-day × 11 days) + (14.3 mg/kg-day × 10 days) + (21.3 mg/kg-day × 12 days)/33 days = 14.8 mg/kg-day.

Table C-41-12. Evidence pertaining to observational immune system effects in animals following exposure to HBCD during development

Reference and study design	Results				
Organ weight					
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning until necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation	**Doses** (mg/kg-d)				
	F1 offspring[a]	**0**	**17**	**168**	**1,570**
	Male, F1	**0**	**11**	**115**	**1,142**
	Female, F1	**0**	**14**	**138**	**1,363**
	F2 offspring[a]	**0**	**15**	**139**	**1,360**
	Absolute spleen weight (mg)				
	Male, F1, adult (n = 22–24)				
	Mean (SD)	885 (168)	840 (147)	878 (163)	851 (113)
	% change[b]	–	−5%	−1%	−4%
	Male, F1, PND 26 (n = 17–23)				
	Mean (SD)	336 (62)	327 (41)	334 (43)	309 (69)
	% change[b]	–	−3%	−1%	−8%
	Female, F1, adult (n = 13–22)				
	Mean (SD)	632 (124)	595 (68)	624 (93)	578 (70)
	% change[b]	–	−6%	−1%	−9%
	Female, F1, PND 26 (n = 14–23)				
	Mean (SD)	311 (53)	306 (44)	304 (59)	280 (40)
	% change[b]	–	−2%	−2%	−10%

Reference and study design	Results				
	Male, F2, PND 26 (n = 13–22)				
	Mean (SD)	360 (83)	361 (54)	346 (78)	263* (50)
	% change[b]	–	0%	–4%	–27%
	Female F2, PND 26 (n = 13–21)				
	Mean (SD)	325 (59)	302 (42)	299 (62)	225* (45)
	% change[b]	–	–7%	–8%	–31%
	Absolute thymus weight (mg)				
	Male, F1, adult (n = 22–24)				
	Mean (SD)	344 (72)	305 (92)	368 (100)	341 (76)
	% change[b]	–	–11%	7%	–1%
	Female, F1, adult (n = 13–22)				
	Mean (SD)	250 (62)	233 (62)	276 (80)	259 (76)
	% change[b]	–	–7%	10%	4%
	Male, F1, PND 26 (n = 17–23)				
	Mean (SD)	342 (68)	339 (50)	369 (59)	317 (57)
	% change[b]	–	–1%	8%	–7%
	Female, F1, PND 26 (n = 14–23)				
	Mean (SD)	335 (64)	330 (58)	370 (58)	305 (31)
	% change[b]	–	–1%	10%	–9%
	Male, F2, PND 26 (n = 13–22)				
	Mean (SD)	343 (92)	336 (57)	360 (88)	282 (71)
	% change[b]	–	–2%	5%	–18%
	Female, F2, PND 26 (n = 13–22)				
	Mean (SD)	338 (85)	324 (50)	331 (69)	260* (80)
	% change[b]	–	–4%	–2%	–23%

Reference and study design	Results								
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11	**Doses** (mg/kg-d)								
		0	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	Absolute popliteal lymph node weight (mg)								
	Male, F1 (n = 4–5)**								
	Mean (SD)	9 (2)	10 (3)	9 (4)	15 (11)	9 (3)	8 (1)	10 (5)	21 (16)
	% change[b]	–	11%	0%	67%	0%	–11%	11%	133%
	Female, F1 (n = 4–5)								
	Mean (SD)	8 (2)	9 (2)	9 (2)	8 (2)	8 (2)	8 (2)	9 (1)	7 (2)
	% change[b]	–	12%	12%	0%	0%	0%	12%	–12%
	Absolute spleen weight (g)								
	Male, F1 (n = 4–5)								
	Mean (SD)	0.49 (0.12)	0.53 (0.07)	0.49 (0.03)	0.58 (0.07)	0.49 (0.05)	0.50 (0.07)	0.58 (0.09)	0.48 (0.06)
	% change[b]	–	8%	0%	18%	0%	2%	18%	–2%
	Female, F1 (n = 4–5)								

Reference and study design	Results								
	Mean (SD)	0.40 (0.04)	0.39 (0.04)	0.37 (0.06)	0.56 (0.37)	0.56 (0.42)	0.38 (0.05)	0.40 (0.04)	0.39 (0.07)
	% change[b]	–	–3%	–8%	40%	40%	–5%	0%	–3%
	Absolute thymus weight (g)								
	Male, F1 (n = 4–5)**								
	Mean (SD)	0.62 (0.10)	0.54 (0.12)	0.53 (0.12)	0.56 (0.13)	0.50 (0.09)	0.55 (0.08)	0.48 (0.14)	0.45 (0.06)
	% change[b]	–	–13%	–15%	–10%	–19%	–11%	–23%	–27%
	Female, F1 (n = 4–5)**								
	Mean (SD)	0.49 (0.07)	0.41 (0.05)	0.40 (0.04)	0.42 (0.05)	0.48 (0.10)	0.45 (0.06)	0.44 (0.11)	0.37 (0.07)
	% change[b]	–	–16%	–18%	–14%	–2%	–8%	–10%	–24%

Reference and study design	Results				
{Hachisuka, 2010, 2919532@@author-year} Rats, SD:IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk recovery period through PNW 11 Only males evaluated	Doses (mg/kg-d)[c]	**0**	**15**	**146**	**1,505**
	Absolute spleen weight (g)				
	Male, F1, PNW 3 (n = 10)				
	Mean (SD)	0.29 (0.05)	0.25 (0.03)	0.22 (0.04)	0.23 (0.04)
	% change[b]	–	–14%	–24%	–21%
	Male, F1, PNW 11				
	Mean (SD)	0.55 (0.08)	0.55 (0.11)	0.56 (0.08)	0.53 (0.13)
	% change[b]	–	0%	2%	–4%
	Absolute thymus weight (g)				
	Male, F1, PNW 3 (n = 10)				
	Mean (SD)	0.21 (0.06)	0.24 (0.05)	0.21 (0.06)	0.21 (0.03)
	% change[b]	–	14%	0%	0%
	Male, F1, PNW 11 (n = 10)				
	Mean (SD)	0.79 (0.08)	0.88 (0.17)	0.88 (0.18)	0.81 (0.13)
	% change[b]	–	11%	11%	3%
Hematology					
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: maternal exposure throughout gestation/lactation;	Doses (mg/kg-d)				
	Male, F1	**0**	**11**	**115**	**1,142**
	Female, F1	**0**	**14**	**138**	**1,363**
	Lymphocyte fraction (%)				
	Male, F1 (n = 10)				
	Mean (SD)	88.2 (4.4)	90.9 (2.7)	87.7 (5.9)	87.3 (5.7)
	% change[b]	–	3%	–1%	–1%
	Female, F1 (n = 10)				
	Mean (SD)	83.6 (9.4)	76.2 (9.6)	83.6 (8.3)	73 (11.6)
	% change[b]	–	–9%	0%	–13%

ED_005297A_00166423-00060

Reference and study design	Results								
dietary exposure post weaning until necropsy									
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11 Only males evaluated	Doses (mg/kg-d)								
		0	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	Basophil cell count in blood ($\times 10^9$/L)								
	Male, F1 (n = 3–4)**								
	Mean (SD)	0.040 (0.004)	0.072 (0.016)	0.063 (0.026)	0.057 (0.016)	0.045 (0.016)	0.048 (0.028)	0.068 (0.008)	0.035 (0.030)
	% change[b]	–	80%	57%	43%	12%	20%	70%	−12%
	Lymphocyte cell fraction in blood (%)								
	Male, F1 (n = 3–4)**								
	Mean (SD)	89.64 (0.29)	89.87 (0.26)	89.45 (0.29)	89.72 (0.18)	88.61 (0.4)	89.61 (0.25)	88.65 (0.15)	85.9 (0.23)
	% change[b]	–	0%	0%	0%	−1%	0%	−1%	−4%
	WBC count in blood ($\times 10^9$/L)								
	Male, F1 (n = 3–4)**								
	Mean (SD)	5.10 (1.01)	7.18 (1.44)	5.72 (1.79)	6.53 (0.72)	4.90 (1.71)	5.92 (2.27)	6.55 (0.14)	4.05 (1.50)
	% change[b]	–	41%	12%	28%	−4%	16%	28%	−21%
{Hachisuka, 2010, 2919532@@author-year} Rats, SD:IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk recovery period through PNW 11 Only males evaluated	Doses (mg/kg-d)[c]								
		0	**14.8**	**146.3**	**1,505**				
	Activated T cell fraction in blood (%)								
	Male, F1, PNW 3 (n = 10)								
	Mean (SD)	13.51 (3.47)	14.01 (2.16)	11.81 (1.96)	10.40* (2.02)				
	% change[b]	–	4%	−13%	−23%				
	Male, F1, PNW 11 (n = 10)								
	Mean (SD)	1.45 (0.54)	1.35 (0.6)	1.27 (0.47)	1.32 (0.24)				
	% change[b]	–	−7%	−12%	−9%				
	Lymphocyte fraction in blood (%)								
	Male, F1, PNW 3 (n = 9–10)								
	Mean (SD)	78.88 (4.74)	79.02 (3.18)	81.69 (3.81)	81.41 (4.06)				
	% change[b]	–	0%	3%	3%				
	Male, F1, PNW 11 (n = 10)								
	Mean (SD)	84.64 (5.46)	84.27 (4.88)	87.56 (4.33)	86.44 (3.36)				
	% change[b]	–	0%	3%	2%				
	NK cell fraction in blood (%)								
	Male, F1, PNW 3 (n = 10)								
	Mean (SD)	0.12 (0.03)	0.1 (0.03)	0.09 (0.02)	0.08* (0.04)				
	% change[b]	–	−17%	−25%	−33%				

Reference and study design	Results								
	Male, F1, PNW 11 (n = 10)								
	Mean (SD)	0.27 (0.07)	0.23 (0.08)	0.27 (0.07)	0.25 (0.09)				
	% change[b]	–	−15%	0%	−7%				
	WBC count in blood ($\times 10^2/\mu L$)								
	Male, F1, PNW 3 (n = 10)								
	Mean (SD)	35.3 (11.3)	30.9 (10)	47.5* (11.8)	39.6 (7.9)				
	% change[b]	–	−12%	35%	12%				
	Male, F1, PNW 11 (n = 10)								
	Mean (SD)	82.1 (17.8)	109.8* (30.8)	110* (29.3)	103.4 (34.1)				
	% change[b]	–	34%	34%	26%				
Histopathology									
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11	**Male, F1** **Female, F1**	**0**	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	WBC count in bone marrow ($\times 10^9$/L)								
	Male, F1 (n = 3–4)**								
	Mean (SD)	9.3 (3.4)	15.0 (9.3)	17.4 (8.5)	13.0 (3.0)	17.9 (4.2)	20.2 (4.1)	16.3 (5.0)	17.6 (4.8)
	% change[b]	–	61%	87%	40%	92%	117%	75%	89%
	CD161a (NK) subpopulation fraction in spleen (%)								
	Male, F1 (n = 3–5)**								
	Mean (SD)	7.9 (0.4)	8.8 (0.8)	8.6 (1.4)	8.9 (1.3)	9.6 (0.6)	8.9 (0.8)	9.0 (1.5)	11.3 (1.3)
	% change[a]	–	11%	9%	13%	22%	13%	14%	43%
	Splenic marginal zone enlargement (incidence)								
	Male, F1 (n = 8–10)								
	Incidence	1/8	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]	7/10*
{Hachisuka, 2010, 2919532@@author-year} Rats, SD:IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk recovery period through PNW 11	**Doses** (mg/kg-d)[c]								
	Male, F1 **Female, F1**	**0**	**15**	**146**	**1,505**				
	CD4NKT (NK) cell fraction in spleen (%)								
	Male, F1, PNW 3 (n = 10)								
	Mean (SD)	6.47 (0.61)	6.28 (0.81)	6.4 (1.31)	5.63* (0.81)				
	% change[b]	–	−4%	−1%	−13%				
	Male, F1, PNW 11 (n = 10)								
	Mean (SD)	12.53 (1.88)	12.89 (1.85)	13.78 (2.66)	13.09 (1.72)				
	% change[b]	–	3%	10%	4%				
	CD8+ CD4- (cytotoxic T-cell) cell fraction in spleen (%)								
	Male, F1, PNW 3 (n = 10)								
	Mean (SD)	6.86 (0.95)	8.12 (2.16)	6.99 (1.42)	6.43 (1.44)				
	% change[b]	–	28%	10%	1%				

Reference and study design	Results				
	Male, F1, PNW 11 (n = 10)				
	Mean (SD)	14.42 (2.23)	18.54* (4.34)	16.85 (4.31)	18.87* (4.82)
	% change[b]	–	29%	17%	31%
	N NKRP1A+CD4- (NK) cell fraction in spleen (%)				
	Male, F1, PNW 3 (n = 10)				
	Mean (SD)	5.75 (0.35)	6.06 (1.09)	5.65 (0.87)	5.09* (0.76)
	% change[b]	–	5%	−2%	−11%
	Male, F1, PNW 11 (n = 10)				
	Mean (SD)	10.63 (1.63)	9.97 (3.44)	11.38 (2.47)	9.44 (2.39)
	% change[b]	–	−6%	7%	−11%
	Activated T-cell fraction in thymus (%)				
	Male, F1, PNW 3 (n = 10)				
	Mean (SD)	2.67 (0.87)	2.46 (0.80)	1.82* (0.55)	1.87 (1.15)
	% change[b]	–	−4%	−29%	−27%
	Male, F1, PNW 11 (n = 10)				
	Mean (SD)	0.92 (0.97)	0.74 (0.51)	1.02 (0.84)	1.04 (0.70)
	% change[b]	–	−20%	11%	13%
	Increased starry sky appearance in thymus				
	Male, F1, PNW 3 (n = 10)				
	Incidence	0/10	0/10	4/10*	1/10
	Male, F1, PNW 11 (n = 10)				
	Incidence	0/10	0/10	0/10	0/10
	Female, F1, PNW 3 (n = 10)				
	Incidence	0/10	0/10	0/10	0/10
	Female, F1, PNW 11 (n = 10)				
	Incidence	0/10	0/10	3/10	0/10
	NK cell fraction in thymus (%)				
	Male, F1, PNW 3 (n = 10)				
	Mean (SD)	0.07 (0.03)	0.07 (0.03)	0.06 (0.02)	0.07 (0.05)
	% change[b]	–	0%	−43%	0%
	Male, F1, PNW 11 (n = 10)				
	Mean (SD)	0.2 (0.04)	0.2 (0.05)	0.25 (0.09)	0.27* (0.08)
	% change[b]	–	0%	25%	35%
	Treg cell fraction in thymus (%)				
	Male, F1, PNW 3 (n = 10)				
	Mean (SD)	7.7 (2.57)	5.15* (0.94)	7.69 (1.27)	7.85 (2.85)
	% change[b]	–	−33%	0%	−5%
	Male, F1, PNW 11 (n = 10)				
	Mean (SD)	4.16 (1.09)	3.98 (0.87)	4.41 (0.76)	4.32 (1.22)
	% change[b]	–	−1%	6%	4%

ED_005297A_00166423-00063

*Statistically significantly different from the control at $p < 0.05$ as reported by study authors.
**Significant dose response trend as reported by study authors.
[a]Percent change compared to control calculated as: (treated value − control value)/control value × 100.
[b]F1 and F2 offspring doses presented as mean maternal gestational F0 and F1 doses, respectively.
[c]TWAs for each exposure group were calculated by: (1) multiplying the measured HBCD intake (mg/kg-day) reported by the study authors for GDs 10–20, PNDs 1–9, and PNDs 9–20 by the number of inclusive days of exposure for each time period; (2) adding the resulting products together; and (3) dividing the sum by the total number of inclusive days (33) of HBCD exposure. Example: 100 ppm = (8.1 mg/kg-day × 11 days) + (14.3 mg/kg-day × 10 days) + (21.3 mg/kg-day × 12 days)/33 days = 14.8 mg/kg-day.
[d]Not measured; only control and high-dose values reported.

Table ~~C-51-13~~. Evidence pertaining to observational immune system effects in animals following exposure to HBCD as adults

Reference and study design	Results				
Organ weight					
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning until necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation	**Doses** (mg/kg-d)				
	Male, F0	**0**	**10**	**101**	**1,008**
	Female, F0	**0**	**14**	**141**	**1,363**
	Absolute spleen weight (mg)				
	Male, F0 (n = 22–24)				
	Mean (SD)	848 (136)	828 (109)	855 (160)	843 (248)
	% change[a]	–	−2%	1%	−1%
	Female, F0 (n = 17–24)				
	Mean (SD)	588 (75)	577 (83)	570 (89)	584 (72)
	% change[a]	–	−2%	−3%	−1%
	Absolute thymus weight (mg)				
	Male, F0 (n = 22–24)				
	Mean (SD)	323 (88)	305 (82)	299 (64)	315 (71)
	% change[a]	–	−6%	−7%	−2%
	Female, F0 (n = 17–24)				
	Mean (SD)	232 (38)	238 (63)	252 (73)	200 (64)
	% change[a]	–	3%	9%	−14%

Reference and study design	Results								
{van der Ven, 2006, 787745@@author-year} Rats, Wistar Gavage 28-d exposure starting on PNW 11	Doses (mg/kg-d)	0	0.3	1	3	10	30	100	200
	Absolute spleen weight (g)								
	Male (n = 4–5)								
	Mean (SD)	0.51 (0.09)	0.59 (0.13)	0.78 (0.55)	0.52 (0.05)	0.58 (0.08)	0.47 (0.03)	0.49 (0.05)	0.50 (0.10)
	% change[a]	–	16%	53%	2%	14%	–8%	–4%	–2%
	Female (n = 4–5)**								
	Mean (SD)	0.41 (0.04)	0.37 (0.04)	0.38 (0.06)	0.44 (0.01)	0.40 (0.04)	0.49 (0.08)	0.53 (0.04)	0.37 (0.05)
	% change[a]	–	–10%	–7%	7%	–2%	20%	29%	–10%
	Absolute thymus weight (g)								
	Male (n = 4–5)**								
	Mean (SD)	0.47 (0.08)	0.45 (0.08)	0.52 (0.17)	0.47 (0.07)	0.50 (0.09)	0.37 (0.06)	0.42 (0.09)	0.38 (0.13)
	% change[a]	–	–4%	11%	0%	6%	–21%	–11%	–19%
	Female (n = 4–5)								
	Mean (SD)	0.42 (0.06)	0.28 (0.10)	0.36 (0.09)	0.35 (0.07)	0.44 (0.07)	0.43 (0.08)	0.42 (0.08)	0.37 (0.10)
	% change[a]	–	–33%	–14%	–17%	5%	2%	0%	–12%

Hematology

Reference and study design	Results				
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: maternal exposure throughout gestation/lactation; dietary exposure post weaning until necropsy	Doses (mg/kg-d)				
	Male, F0	**0**	**10**	**101**	**1,008**
	Female, F0	**0**	**14**	**141**	**1,363**
	Lymphocyte fraction (%)				
	Male, F0 (n = 10)				
	Response	88.5 (6.5)	88.8 (2.4)	88.8 (3.9)	87.5 (4.6)
	% change[a]	–	0%	0%	–1%
	Female, F0 (n = 10)				
	Mean (SD)	72.5 (8.7)	85* (5)	78.4 (9.5)	70.8 (9)
	% change[a]	–	17%	8%	–2%
	Segmented neutrophil fraction (%)				
	Male, F0 (n = 10)				
	Mean (SD)	8.00 (5.24)	8.24 (1.98)	7.68 (3.26)	8.68 (4.61)
	% change[a]	–	3%	–4%	8%
	Female, F0 (n = 10)				
	Mean (SD)	21.68 (8.08)	10.56* (4.19)	16.84 (9.19)	23.28 (8.13)
	% change[a]	–	–51%	–22%	7%
	Stab form neutrophil fraction (%)				
	Male, F0 (n = 10)				
	Mean (SD)	0.48 (0.73)	0.36 (0.3)	0.64 (0.28)	0.56 (0.51)

Reference and study design	Results								
	% change[a]	–	–25%	33%	17%				
	Female, F0 (n = 10)								
	Mean (SD)	1.32 (0.57)	0.60* (0.39)	0.84 (0.55)	1.12 (0.7)				
	% change[a]	–	–55%	–36%	–15%				
{van der Ven, 2006, 787745@@author-year} Rats, Wistar Gavage 28-d exposure starting on PNW 11	**Doses** (mg/kg-d)								
	Male	**0**	**0.3**	**1**	**3**	**10**	**30**	**100**	**200**
	Lymphocyte cell fraction in blood (%)								
	Male (n = 3–5)								
	Mean (SD)	89.1 (2.5)	89.0 (3.7)	85.4 (5.9)	85.3 (2.0)	86.7 (3.7)	88.9 (3.8)	84.2 (8.1)	88.1 (3.1)
	% change[a]	–	0%	–4%	–4%	–3%	0%	–5%	–1%
Histopathology									
{van der Ven, 2006, 787745@@author-year} Rats, Wistar Gavage 28-d exposure starting on PNW 11	**Doses** (mg/kg-d)								
		0	**0.3**	**1**	**3**	**10**	**30**	**100**	**200**
	CD4 (Th) cells per spleen (cells $\times 10^7$)								
	Male (n =1–5)**								
	Mean (SD)	14.0 (4.7)	15.2 (n/a)	13.3 (4.8)	11.4 (n/a)	10.5 (0.9)	9.0 (n/a)	11.2 (n/a)	10.0 (2.0)
	% change[a]	–	9%	–5%	–19%	–25%	–36%	–20%	–29%
	Total immune cells per spleen (cells $\times 10^7$)								
	Male (n =1–5)**								
	Mean (SD)	48.7 (10.5)	49.6 (n/a)	47.1 (15.4)	44.4 (n/a)	39.4 (3.8)	29.7 (n/a)	37.0 (n/a)	35.8 (1.1)
	% change[a]	–	2%	–3%	–9%	–19%	–39%	–24%	–26%

*Statistically significantly different from the control at $p < 0.05$ as reported by study authors.
**Significant dose response trend as reported by study authors.
[a]Percent change compared to control calculated as: (treated value – control value)/control value × 100

ED_005297A_00166423-00066

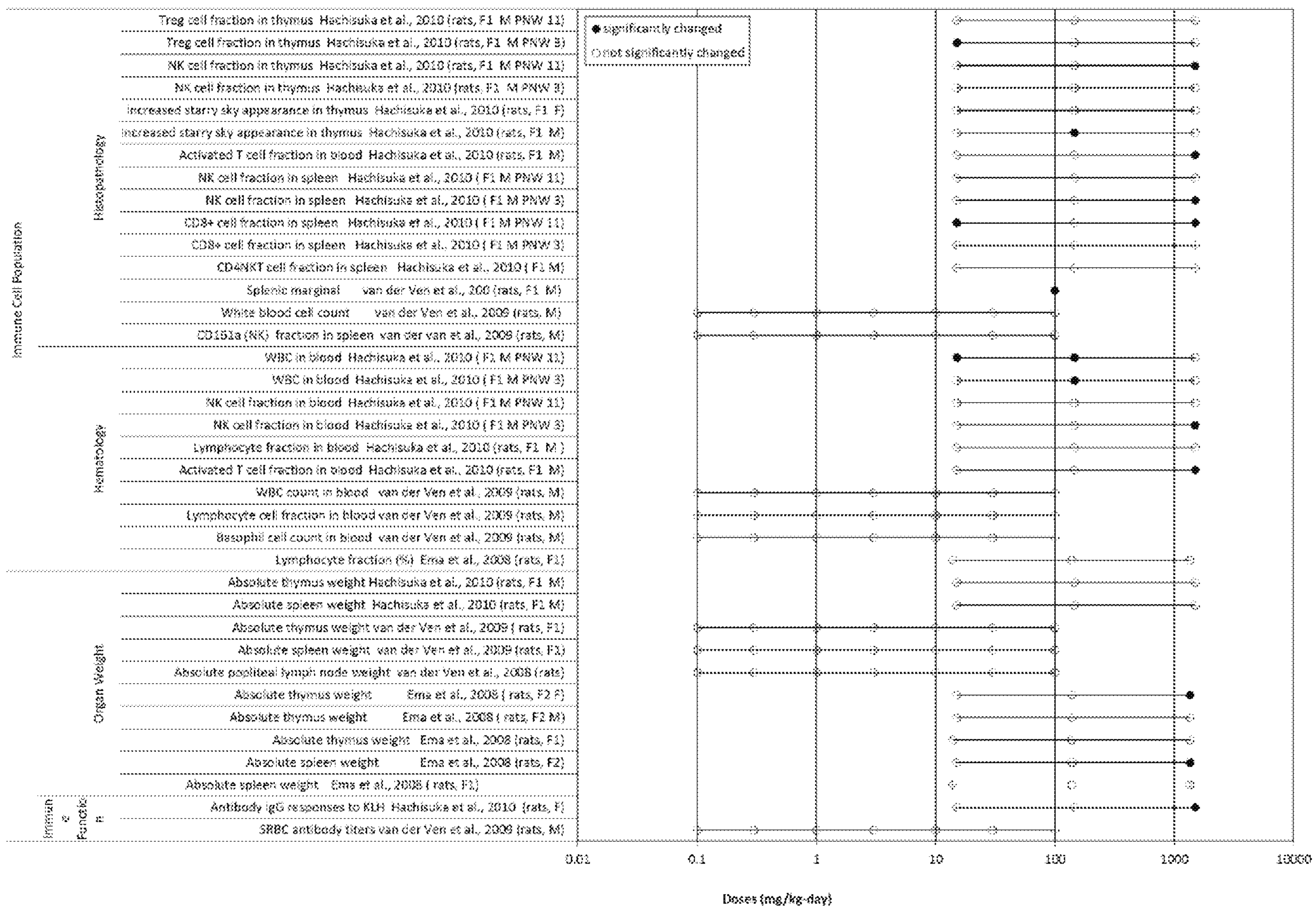


Figure 1-10. Exposure response array of immune system following oral exposure.

Commented [LA7]: New ER arrays are housed in HAWC.

ED_005297A_00166423-00067

Mechanistic Evidence

Mechanistic information to support HBCD-mediated effects on the immune system is limited. Several recent in vitro studies in human immune cells suggest that HBCD may alter immune function through activation of MAPK signaling pathways (ERK1/2 and p38) resulting in increased secretion of IFN γ and IL-1β, pro-inflammatory cytokines that regulate immune function (Almighamsi, 2016; Anisuzzman, 2016; Cato, 2016). Similarly, pro-inflammatory effects driven by were observed in human brochial epithelial cells (BEAS-2B); HBCD exposure increased expression of proinflammatory cytokines (IL-6 and IL-8) and ICAM-1, a cell surface marker often expressed by immune cells, which were mediated by activation of MAPK signaling pathways (Koike, 2016). One study using human monocyte-derived dendritic cells found that co-exposure with HBCD enhanced IL-6 and IL-8 secretion elicited by environmental allergens (Canbaz, 2016).

{Koike, 2012, 1400827@@author-year} used bone marrow-derived dendritic cells prepared from atopic-prone NC/Nga mice to investigate HBCD effects on the immune response in vitro. HBCD (10 μg/mL) increased cell proliferation and expression of a dendritic activation marker, DEC205. Bone marrow-derived dentritic cells differentiated in the presence of HBCD also showed enhanced MHC class II, CD80, CD86, and CD11c expression. These in vitro data are supported by two studies using the guinea pig maximization test method that indicated that HBCD may act as a mild skin allergen {Momma, 1993, 1927836;Nakamura, 1994, 1928219}. Taken together, these studies suggest that HBCD may stimulate an immune response by increasing the activity of antigen-presenting cells. In vitro, HBCD altered several aspects of human NK cell function, including decreased target cell binding, expression of surface binding proteins, lytic function, and ATP levels {Hinkson, 2009, 1927711;Hinkson, 2010, 1927693}; however, in vivo NK cell activity was unaffected in rats {van der Ven, 2009, 589273;van der Ven, 2006, 787745}.

Integration of Evidence

The potential immunotoxicity of HBCD has not been investigated in human populations. The effects of HBCD on both functional and structural immune endpoints were evaluated in animal models. Of the endpoints evaluated, measures of T cell-dependent antibody responses—functional immune endpoints and therefore more sensitive and predictive indicators of potential immunotoxicity {Luster, 2005, 2174509}—were given more weight. In studies in rats, early-life HBCD exposure altered antibody responses to sheep red blood cells (SRBC) (increased) {van der Ven, 2009, 589273} and keyhole limpet hemocyanin (KLH) (decreased) {Hachisuka, 2010, 2919532}. Healthy immune function is maintained as a delicate balance between: (1) an immune response adequate to provide protection from certain types of cancers and infectious diseases, and (2) pathological loss of immune system control resulting in conditions such as autoimmunity, hypersensitivity, and chronic inflammation. Unintended immunomodulation in either direction (i.e., immunosuppression or immunostimulation) may be considered adverse {WHO, 2012, 1249755}. Therefore, the difference in direction of effect in the only two measures of antibody response does not necessarily minimize the validity of the findings in early lifestage animals. HBCD did not cause changes in functional immune endpoints in adult rats or mice {van der Ven, 2006, 787745;Watanabe, 2010, 1927692}. The database does not provide a clear and consistent pattern of effect on immune organ weights, hematology, or histopathology. Given the diversity of study designs, exposure conditions, and analytical methods represented in this database, it is difficult to identify underlying reason(s) for the differences in observations across studies. Overall, there is inadequate information to assess immune system toxicity following exposure to HBCD (See also Section 1.2.6 of the Toxicological Review).

C.2.3 Genotoxicity Information

A limited number of studies have investigated the genotoxicity of HBCD; these are summarized in Table C-6~~1~~. The majority of these studies were standard Ames tests for detecting mutagenic potential in *Salmonella typhimurium*. These tests, which employ different strains of bacteria that have been developed with pre-existing mutations, including *S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538, are referred to as reversion assays {Maron, 1983, 195187}. Most of these assays conducted with HBCD yielded negative results {IBT Labs, 1990, 787688;Litton Bionetics, 1990, 787698;SRI International, 1990, 787716;Zeiger, 1987, 699386;Huntingdon Research Centre, 1990, 787683;Pharmakologisches Inst, 1990, 787701}. Among the few assays performed to determine the genotoxicity of HBCD in prokaryotic systems, one in yeast {Litton Bionetics, 1990, 787698}, one detecting chromosomal aberrations in human peripheral lymphocytes in vitro {Microbiological Associates, 1996, 787699}, and one in vivo mouse micronucleus test following intraperitoneal (i.p.) injections of HBCD {BASF, 2000, 787637} were negative, even when tested at cytotoxic concentrations.

Table C-6~~1~~. Summary of genotoxicity studies of HBCD

Test/species/strain/ route	Test doses (per plate)[a]	Results[b]		Notes	Reference
		–S9	+S9		
Eukaryotic systems, in vitro					
S. typhimurium TA98, TA100, TA1535, TA1537	50–5,000 μg (HBCD bottoms) in acetone	+ (TA1535 and 100 only)	+ (TA100 only)	No cytotoxicity observed. Dose-response observed in TA1535 (–S9) ≥100 μg/plate. TA100 positive at highest dose only (5,000 μg/plate). All doses had a black precipitate thought to be carbon.	{Ethyl Corporation, 1990, 787661@@author-year}

Test/species/strain/ route	**Test doses (per plate)[a]**	**Results[b]**		**Notes**	**Reference**
		–S9	**+S9**		
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	50 µg (421–32B) (solvent not reported)	–	–		{Litton Bionetics, 1990, 787698@@author-year}
S. typhimurium TA98, TA100, TA1535, TA1537	2–1,000 µg (GLS-S6-41A) in DMSO	–	–		{GSRI, 1978, 1937197@@author-year}
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	100–10,000 µg in DMSO	–	–	Doses ≥1,000 µg were insoluble.	{Zeiger, 1987, 699386@@author-year}
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	250 µg (Firemaster, FM-100, Lot 53, white powder) in DMSO	–	–	Doses ≥250 µg were insoluble.	{IBT Labs, 1990, 787688@@author-year}
	1,000 µg (FM-100, Lot 3322, liquid residue) in DMSO	–	+ (TA1535 only)	Significant in TA1535 at highest dose only.	
S. typhimurium TA98, TA100, TA1537	3,000 µg in DMSO	–	–	Doses ≥1,000 µg were partially insoluble.	{Pharmakologisches Inst, 1990, 787701@@author-year}
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	5,000 µg in DMSO	–	–	No cytotoxicity observed.	{SRI International, 1990, 787716@@author-year}
S. typhimurium TA92, TA94, TA98, TA100, TA1535, TA1537	10,000 µg (Pyroguard SR-103) in DMSO	–	–		{Ogaswara, 1993, 2344713@@author-year}
S. typhimurium TA98, TA100, TA1535	10,000 µg in DMSO	–	–	Insoluble at 10,000 µg.	{Huntingdon Research Centre, 1990, 787683@@author-year}
Prokaryotic non-mammalian systems, in vitro					
Saccharomyces cerevisiae D4	50 µg (solvent not reported)	–	–		{Litton Bionetics, 1990, 787698@@author-year}

Test/species/strain/route	Test doses (per plate)[a]	Results[b]		Notes	Reference
		–S9	+S9		
Mammalian systems, in vivo					
Micronucleus test mouse/NMRI/i.p. injection	2,000 mg/kg in DMSO	– (T)	NA	Toxicity evident as a slight inhibition of erythropoiesis at 2,000 mg/kg. Number of polychromatic erythrocytes with micronuclei from femoral bones evaluated 24 hrs after 2[nd] injection.	{BASF, 2000, 787637@@author-year}
Mammalian systems, in vitro					
Chromosomal aberration test Human peripheral blood lymphocytes	750 μg/mL (–S9) 250 μg/mL (+S9) in DMSO	– (T)	– (T)	Doses 750–2,500 μg/mL were partially insoluble, and fully insoluble >2,500 μg/mL. Repeated test for two harvest time points: 20-hr (–S9) or 4-hr (+S9) incubations, and 20- or 44-hr incubations (–S9 and +S9).	{Microbiological Associates, 1996, 787699@@author-year}
Reversion assay CHO/V79/Sp5 and SPD8 Intragenic recombination at *hprt* locus in Sp5 (non-HR) and SPD8 (HR) duplication cell lines	3–20 μg/mL in DMSO	+	NA	A statistically significant, dose-dependent increase in reversion frequency was observed in both assays as determined by linear regression analysis. Significant inhibition of cloning efficiency occurred at doses ≥15 μg/mL in the SPD8 assay and ≥20 μg/mL in the Sp5 assay. Cytotoxicity (IC_{50}) measured at 0.02–0.03 mM.	{Helleday, 1999, 787680@@author-year}
Unscheduled DNA synthesis rat/F344 male/primary hepatocytes	10 μg/well in acetone (HBCD bottoms)	+	NA	Five highest doses (from 5 μg/well) showed an increased response with dose over solvent control, but only four highest were statistically significant (χ^2). Highest dose (1,000 μg/well) was cytotoxic.	{Ethyl Corporation, 1990, 1928253@@author-year}

[a]Lowest effective dose for positive results; highest dose tested for negative results.

ED_005297A_00166423-00071

[b]+ = positive; ± = equivocal or weakly positive; − = negative; T = cytotoxicity; NA = not applicable.

DMSO = dimethyl sulfoxide

Some positive results have been reported. *S. typhimurium* strain TA1535 was positive for reverse mutations at the highest dose only using a liquid residue of HBCD in DMSO {IBT Labs, 1990, 787688}, and strain TA100 was positive also at the highest dose using an unidentified mixture characterized only as HBCD bottoms in acetone {Ethyl Corporation, 1990, 787661}. In this same study, TA1535 was positive at ≥100 μg/plate without addition of an S9 microsomal fraction {Ethyl Corporation, 1990, 787661}. The number of revertants increased with dose. This was the only Ames study to report dissolving the test article in a solvent other than DMSO (in this case, acetone). DMSO is a free-radical scavenger and can potentially obscure genetic damage due to oxidative radicals. Both strains TA1535 and TA100 were designed to be sensitive to detecting reversions by base substitution, a type of genetic lesion that can result from oxidative DNA damage due to reactive oxygen species (ROS). However, there is only limited evidence in the literature indicating that HBCD exposure may induce oxidative stress {An, 2013, 1927550;Hu, 2009, 837636}.

In mammalian systems, a reverse mutation assay with Chinese hamster ovary (CHO) Sp5 and SPD8 cell lines exposed to HBCD {Helleday, 1999, 787680} yielded positive results. These two clones exhibit a partial duplication of the hprt gene, causing lethality unless a reversion occurs, either via homologous recombination (SPD8) or non-homologous recombination (Sp5). A statistically significant, dose-dependent increase in reversion frequency was observed in both clones, although at higher doses, there was a significant inhibition of cloning efficiency. In addition, a test of unscheduled DNA synthesis with rat hepatocytes exposed to HBCD bottoms was positive {Ethyl Corporation, 1990, 1928253}, and also showed an increase in response with dose.

It is noteworthy that in these three studies {Helleday, 1999, 787680}, the positive results were dose-dependent, observed at nontoxic doses, and in two assays, specific for detecting mutations. However, the Ames tests in the same strains that showed positive results (TA1535 and TA100) were negative in seven other studies, and the results in the reverse mutation assay in CHO cells {Helleday, 1999, 787680} have not been confirmed by another group. Overall, given the negative results in the majority of mutation assays and the negative results in two assays for chromosomal aberrations in mammalian cells {BASF, 2000, 787637;Microbiological Associates, 1996, 787699}, the evidence does not indicate that HBCD is genotoxic.

APPENDIX D. DOSE-RESPONSE MODELING FOR THE DERIVATION OF REFERENCE VALUES FOR EFFECTS OTHER THAN CANCER AND THE DERIVATION OF CANCER RISK ESTIMATES

This appendix provides technical detail on dose-response evaluation and determination of points of departure (PODs) for relevant toxicological endpoints. The endpoints were modeled using the U.S. Environmental Protection Agency (EPA) Benchmark Dose Software (BMDS, version 2.6). This appendix describes the common practices used in evaluating the model fit and selecting the appropriate model for determining the POD, as outlined in the *Benchmark Dose Technical Guidance Document* {U.S. EPA, 2012, 1239433}. In some cases, it may be appropriate to use alternative methods, based on statistical judgment; exceptions are noted as necessary in the summary of the modeling results.

D.1 NONCANCER ENDPOINTS

The noncancer endpoints that were selected for dose-response modeling are presented in Table D-1. For each endpoint, the doses and response data used for the modeling are presented.

Table D-[SEQ Table * ARABIC \s 1]. Noncancer endpoints selected for dose-response modeling for HBCD

Endpoint	Species (strain)/sex	Dose (mg/kg-d)[a]	Incidence [%] or mean ± SD (number of animals or litters)	BMR(s)
Thyroid				
↓T4 {Ema, 2008, 787657@@author-year}	F0 rats (CRL Sprague-Dawley)/male	0 10 101 1,008 *TWA of lifetime exposure, F0*	4.04 ± 1.42 (8) 3.98 ± 0.89 (8) 2.97 ± 0.76 (8) 2.49 ± 0.55 (8)	10% RD, 15% RD, 20% RD, 1 SD
↓T4 {Ema, 2008, 787657@@author-year}	F0 rats (CRL Sprague-Dawley)/female	0 14 141 1,363 *TWA of lifetime exposure, F0*	2.84 ± 0.61 (8) 3.14 ± 0.48 (8) 3.00 ± 0.77 (8) 1.96 ± 0.55 (8)	10% RD, 15% RD, 20% RD, 1 SD

ED_005297A_00166423-00073

Endpoint	Species (strain)/sex	Dose (mg/kg-d)[a]	Incidence [%] or mean ± SD (number of animals or litters)	BMR(s)
↓T4 {Ema, 2008, 787657@@author-year}	F1 rats (CRL Sprague-Dawley)/female	0 14.3 138 1,363 *TWA of lifetime exposure, F1*	3.59 ± 1.08 (8) 3.56 ± 0.53 (8) 3.39 ± 1.21 (8) 2.58 ± 0.37 (8)	10% RD, 15% RD, 20% RD, 1 SD
Liver				
Relative liver weight {Ema, 2008, 787657@@author-year}	F1 rats (CRL Sprague-Dawley)/male weanlings, PND 26	0 16.5 168 1,570 *TWA of F0 gestational and lactational doses*	4.6 ± 0.37 (23) 4.6 ± 0.32 (21) 5.05 ± 0.32 (20) 6 ± 0.44 (17)	10% RD, 1 SD
Relative liver weight {Ema, 2008, 787657@@author-year}	F1 rats (CRL Sprague-Dawley)/female weanlings, PND 26	0 16.5 168 1,570 *TWA of F0 gestational and lactational doses*	4.57 ± 0.35 (23) 4.59 ± 0.28 (21) 5.02 ± 0.32 (20) 6.07 ± 0.36 (14)	10% RD, 1 SD
Relative liver weight {Ema, 2008, 787657@@author-year}	F1 rats (CRL Sprague-Dawley)/male adults	0 11.4 115 1,142 *TWA of lifetime exposure, F1*	3.27 ± 0.18 (24) 3.34 ± 0.26 (24) 3.37 ± 0.25 (22) 3.86 ± 0.28 (24)	10% RD, 1 SD
Relative liver weight {Ema, 2008, 787657@@author-year}	F1 rats (CRL Sprague-Dawley)/female adults	0 14.3 138 1,363 *TWA of lifetime exposure, F1*	4.18 ± 0.42 (22) 4.39 ± 0.44 (22) 4.38 ± 0.47 (20) 5.05 ± 0.50 (13)	10% RD, 1 SD
Relative liver weight {Ema, 2008, 787657@@author-year}	F2 rats (CRL Sprague-Dawley)/male weanlings, PND 26	0 14.7 139 1,360 *TWA of F1 gestational and lactational doses*	4.72 ± 0.59 (22) 4.74 ± 0.35 (22) 5.04 ± 0.4 (18) 6.0 ± 0.25 (13)	10% RD, 1 SD

Endpoint	Species (strain)/sex	Dose (mg/kg-d)[a]	Incidence [%] or mean ± SD (number of animals or litters)	BMR(s)
Relative liver weight {Ema, 2008, 787657@@author-year}	F2 rats (CRL Sprague-Dawley)/female weanlings, PND 26	0 14.7 139 1,360 *TWA of F1 gestational and lactational doses*	4.70 ± 0.27 (21) 4.70 ± 0.28 (22) 4.94 ± 0.32 (20) 5.89 ± 0.44 (13)	10% RD, 1 SD
Relative liver weight {WIL Research, 2001, 787787@@author-year}	Rats (Sprague-Dawley)/male	0 100 300 1,000	2.709 ± 0.1193 (10) 3.175 ± 0.2293 (10) 3.183 ± 0.2653 (10) 3.855 ± 0.1557 (9)	10% RD, 1 SD
Relative liver weight {WIL Research, 2001, 787787@@author-year}	Rats (Sprague-Dawley)/female	0 100 300 1,000	2.887 ± 0.2062 (10) 3.583 ± 0.2734 (10) 3.578 ± 0.3454 (10) 4.314 ± 0.2869 (10)	10% RD, 1 SD
Reproductive				
Primordial follicles {Ema, 2008, 787657@@author-year} (supplemental)	F1 parental rat (CRL Sprague-Dawley)/female	0 9.6 96 941 *The F0 adult female gestational doses*	316.3 ± 119.5 (10) 294.2 ± 66.3 (10) 197.9 ± 76.9 (10) 203.4 ± 79.5 (10)	1% ER, 5% ER, 10% ER
Incidence of non-pregnancy {Ema, 2008, 787657@@author-year}	F0 and F1 parental rats combined (CRL Sprague-Dawley)/female	0 13.3 132 1,302 *TWA F0, F1 female pre-mating doses*	1/48 [2%] 3/48 [6.2%] 7/48 [14.5%] 7/47 [14.9%]	5% ER, 10% ER
Developmental				
Offspring loss at PND 4 {Ema, 2008, 787657@@author-year}	F2 offspring rats (CRL Sprague-Dawley)	0 9.7 100 995 *The F1 adult female gestational doses*	28/132 [21%] 26/135 [19.3%] 23/118 [19.5%] 47/120 [39.2%]	1% ER, 5% ER

Endpoint	Species (strain)/sex	Dose (mg/kg-d)[a]	Incidence [%] or mean ± SD (number of animals or litters)	BMR(s)
Offspring loss at PND 21 {Ema, 2008, 787657@@author-year}	F2 offspring rats (CRL Sprague-Dawley)	0 19.6 179 1,724 *The F1 adult female lactational doses*	11/70 [15.7%] 7/70 [10.0%] 18/64 [28.1%] 32/64 [50.0%]	1% ER, 5% ER
Pup weight during lactation at PND 21 {Ema, 2008, 787657@@author-year}	F2 offspring rats (CRL Sprague-Dawley)/male	0 19.6 179 1,724 *The F1 adult female lactational doses*	53 ± 12.6 (22) 56.2 ± 6.7 (22) 54.1 ± 10.1 (18) 42.6 ± 8.3 (13)	5% RD, 10% RD, 0.5 SD, 1 SD
Pup weight during lactation at PND 21 {Ema, 2008, 787657@@author-year}	F2 offspring rats (CRL Sprague-Dawley)/female	0 19.6 179 1,724 *The F1 adult female lactational doses*	52 ± 10 (21) 52.8 ± 6.6 (22) 51.2 ± 10.8 (20) 41.6 ± 8.4 (13)	5% RD, 10% RD, 0.5 SD, 1 SD

[a]Doses were calculated as TWA doses using weekly average doses (in mg/kg-day) as reported in Table 10 of the Supplemental Materials to {Ema, 2008, 787657@@author-year}.

BMR = benchmark response; ER = extra risk; PND = postnatal day; RD = relative deviation; SD = standard deviation; T4 = thyroxine; TWA = time-weighted average

D.2 DOSE-RESPONSE MODELING FOR NONCANCER ENDPOINTS

D.2.1 Evaluation of Model Fit

For each dichotomous endpoint where only summary data (i.e., number affected and total number exposed per group) were available, BMDS dichotomous models[1] were fitted to the data using the maximum likelihood method. Each model was tested for goodness-of-fit using a chi-square goodness-of-fit test (χ^2 p-value < 0.10 indicates lack of fit). Other factors were also used to assess model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the benchmark response (BMR).

[1]Unless otherwise specified, all available BMDS dichotomous models besides the alternative and nested dichotomous models were fitted. The following parameter restrictions were applied: for the LogLogistic model, restrict slope ≥1; for the Gamma and Weibull models, restrict power ≥1.

ED_005297A_00166423-00076

For each dichotomous endpoint for which incidence data were available for individual animals, BMDS nested dichotomous models[2] were fitted to the data using the maximum likelihood method. Each nested model was tested for goodness-of-fit using a bootstrap approach. Chi-square statistics were computed with both bootstrap iterations and original data. The *p*-value was the proportion of chi-square values from the iterations that were greater than the original chi-square value (χ^2 *p*-value < 0.10 indicates lack of fit). Other factors were also used to assess model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the BMR.

For each continuous endpoint, BMDS continuous models[3] were fitted to the data using the maximum likelihood method. Model fit was assessed by a series of tests as follows. For each model, first the homogeneity of the variances was tested using a likelihood ratio test (BMDS Test 2). If Test 2 was not rejected (χ^2 *p*-value ≥ 0.10), the model was fitted to the data assuming constant variance. If Test 2 was rejected (χ^2 *p*-value < 0.10), the variance was modeled as a power function of the mean, and the variance model was tested for adequacy of fit using a likelihood ratio test (BMDS Test 3). For fitting models using either constant variance or modeled variance, models for the mean response were tested for adequacy of fit using a likelihood ratio test (BMDS Test 4, with χ^2 *p*-value < 0.10 indicating inadequate fit). Other factors were also used to assess the model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the BMR.

D.2.2 Model Selection

To select the appropriate model from which to derive the POD for each endpoint, the BMDL estimate (95% lower confidence limit on the benchmark dose [BMD], as estimated by the profile likelihood method) and Akaike's information criterion (AIC) value were used to select the model from among the models exhibiting adequate fit. If the BMDL estimates were "sufficiently close," that is, differed by at most 3-fold, the model selected was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, the lowest BMDL was selected as the POD.

For nested dichotomous models, there are the options of including a litter-specific covariate and estimating intralitter correlations, yielding four combinations of option selections, as displayed in [REF _Ref390862895 \h * MERGEFORMAT]. All the three nested dichotomous models were fitted for every combination in the table, yielding four sets of models (12 model runs in total).

---

[2]Unless otherwise specified, all available BMDS nested dichotomous models were fitted. For the nested Logistic, NCTR, and Rai and van Ryzin models, power ≥1 was applied.
[3]Unless otherwise specified, all available BMDS continuous models were fitted. The following parameter restrictions were applied: for the polynomial models, restrict the coefficients b1 and higher to be nonnegative or nonpositive if the direction of the adverse effect is upward or downward, respectively; for the Hill, Power, and Exponential models, restrict power ≥1.

ED_005297A_00166423-00077

Table D-[SEQ Table * ARABIC \s 1]. The combinations of option selections for the nested dichotomous models

Litter-specific covariates used Intralitter correlations estimated	Litter-specific covariates used Intralitter correlations assumed zero
Litter-specific covariates not used Intralitter correlations estimated	Litter-specific covariates not used Intralitter correlations assumed zero

The appropriate model was selected from this set of 12 models using the same procedure as for the non-nested models as described in Section 2.3.9 (page 39) of the *Benchmark Dose Technical Guidance Document* {U.S. EPA, 2012, 1239433}. If multiple litter specific covariates were tested, this same set of 12 modeling options was evaluated for each litter-specific covariate (e.g., litter size, implantation site, dam body weight) and the appropriate model was selected from the expanded set of modeling options (12 × number of litter-specific covariates considered) using the same procedure as for the non-nested models.

D.2.3 Modeling Results

Below are tables summarizing the modeling results for the noncancer endpoints modeled.

ED_005297A_00166423-00078

D.2.3.1 *Thyroid*

Table D-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for T4 in F0 parental male CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}; BMR = 10% RD from control mean, 15% RD from control mean, 20% RD from control mean, and 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{15RD} (mg/kg-d)	$BMDL_{15RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2) Exponential (M3)[b]	0.0473	33.926	259	177	399	274	Of the models without saturation that provided an adequate fit and a valid BMDL estimate, the ~~Hill~~ Exponential 4 model with modeled variance was selected based on lowest AIC (BMDLs differed by <3).
Exponential (M4) **Exponential (M5)[c]**	**0.742**	**29.933**	**23.9**	**6.99**	**39.1**	**11.5**	
Hill	0.949	29.829	14.4	3.21	25.6	5.66	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.0418	34.174	303	227	455	341	
Model[a]	**Goodness of fit**		**BMD_{20RD} (mg/kg-d)**	**$BMDL_{20RD}$ (mg/kg-d)**	**BMD_{1SD} (mg/kg-d)**	**$BMDL_{1SD}$ (mg/kg-d)**	
	***p*-value**	**AIC**					
Exponential (M2) Exponential (M3)[b]	0.0473	33.926	548	376	866	511	
Exponential (M4) **Exponential (M5)[c]**	**0.742**	**29.933**	**57.9**	**17.2**	**101**	**29.5**	
Hill	0.949	29.829	42.0	9.11	94.9	Error[g]	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.0418	34.174	607	454	906	595	

Commented [LA8]: Model selection changed from the prvious draft (from the Hill model to the Exp4 model).

[a]Modeled variance case presented (BMDS Test 2 *p*-value = 0.0756, BMDS Test 3 *p*-value = 0.553), selected model in bold; scaled residuals for selected model for doses 0, 10.2, 101, and 1,008 mg/kg-day were -0.1665~~-0.309~~, 0.166~~0.349~~, 0.03642~~0.0059~~, and -0.03619, ~~-0.0466,~~ respectively.

[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

[c]For the Exponential (M5) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M4) model.

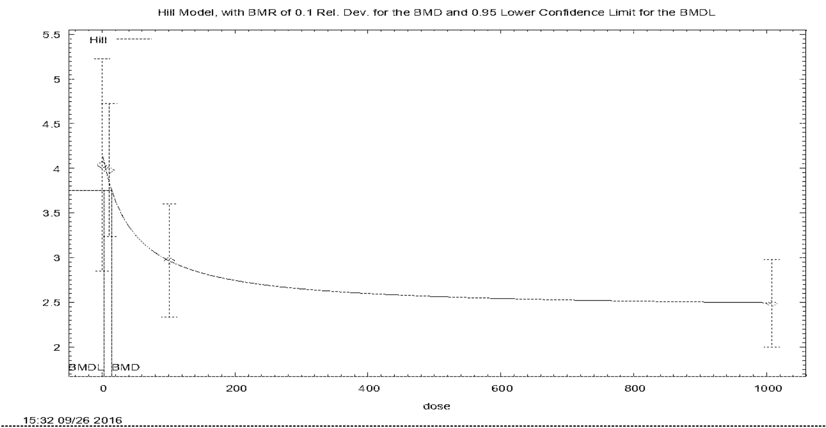
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.

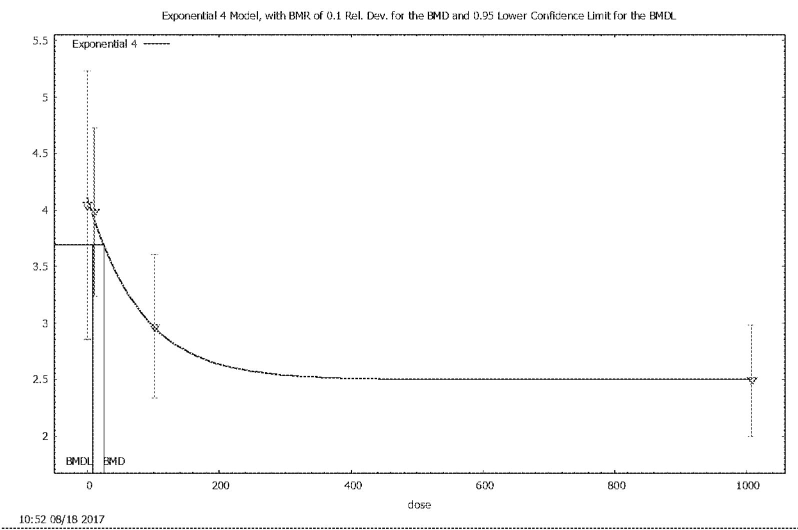
[e]For the Polynomial 3° model, the b3 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.

[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

[g]BMD or BMDL computation failed for this model.

ED_005297A_00166423-00079





BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for ~~Hill~~ Exponential 4 Model, for T4 in F0 parental CRL Sprague-Dawley male rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential 4 Model (Version: 1.10~~2.17~~; Date: 01/12~~28~~/2015~~3~~)

The form of the response function is:

Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]~~Y[dose] = intercept + v*dose^n/(k^n + dose^n)~~

A modeled variance is fit

ED_005297A_00166423-00080

Benchmark Dose Computation

BMR = 10% RD

BMD = ~~1~~23.894~~64.4043~~

BMDL at the 95% confidence level = 6.99406~~3.21225~~

Parameter Estimates

Variable	Estimate	Default initial parameter values
lalpha	-3.94284 ~~-4.00393~~	-3.54227 ~~-0.0687608~~
rho	2.98463 ~~3.0323~~	2.72754~~0~~
~~intercept~~a	4.1075 ~~4.16873~~	4.242~~4.04~~
~~v~~b	0.0123219 ~~-1.74587~~	0.00282274~~-1.55~~
~~n~~c	~~1~~0.607906	0.559035~~2.12371~~
d~~k~~	~~45.9212~~1 (specified)	~~74.4792~~1 (specified)

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	4.04	4.11~~4.17~~	1.42	1.15~~1.18~~	-0.167 ~~-0.309~~
10.2	8	3.98	3.92~~3.85~~	0.89	1.07~~1.04~~	0.166~~0.349~~
101	8	2.97	2.961~~2.97~~	0.76	0.71~~0.7~~	0.036~~0.0059~~
1,008	8	2.49	2.50~~2.5~~	0.59	0.56~~0.54~~	-0.036~~-0.0466~~

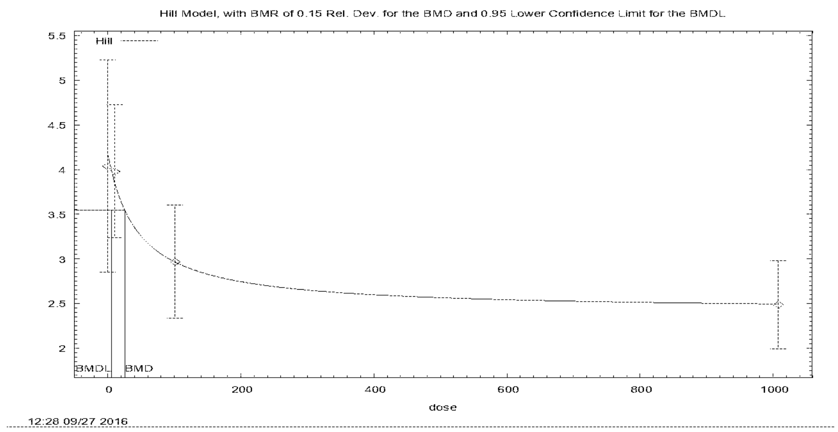
Likelihoods of Interest

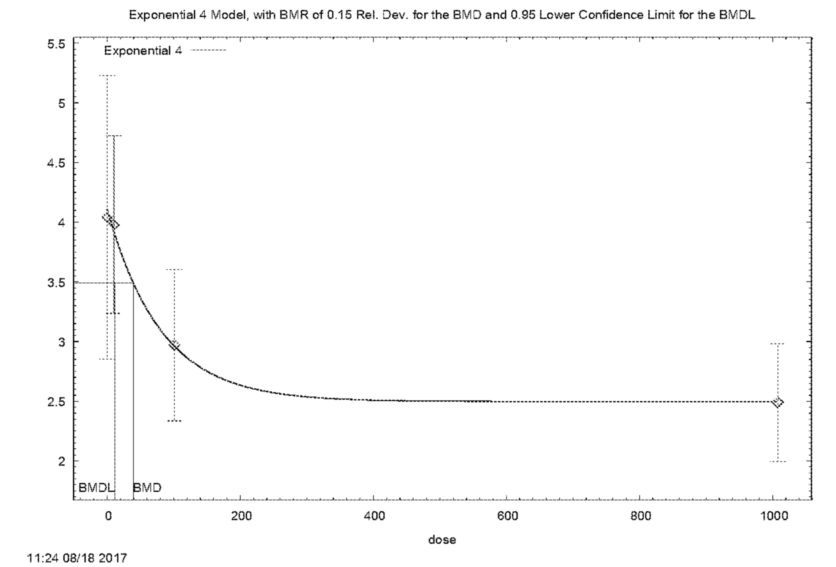
Model	Log (likelihood)	Number of parameters	AIC
A1	-12.76333 ~~-12.763326~~	5	35.52665~~35.526651~~
A2	-9.319925~~-9.319925~~	8	34.63985~~34.639851~~
A3	-9.91228~~-9.91228~~	6	31.82456~~31.82456~~
fitted	-9.966286 ~~-9.914356~~	5	29.93257~~29.828712~~
R	-19.64317~~-19.643171~~	2	43.28634~~43.286341~~

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	20.65 ~~20.6465~~	6	0.002123
Test 2	6.887~~6.8868~~	3	0.07559
Test 3	1.185~~1.18471~~	2	0.553
Test 6a~~4~~	0.108 ~~0.00415236~~	1	0.7424~~0.9486~~

df = degree(s) of freedom





BMR = 15% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for ~~Hill~~ Exponential 4 Model, for T4 in F0 parental CRL Sprague-Dawley male rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

~~**Hill Model** (Version: 2.17; Date: 01/28/2013)~~
~~The form of the response function is: Y[dose] = intercept + v*dose^n/(k^n + dose^n)~~
~~A modeled variance is fit~~

~~**Benchmark Dose Computation**~~
~~BMR = 15% RD~~
~~BMD = 25.6254~~
~~BMDL at the 95% confidence level = 5.6584~~

~~**Parameter Estimates**~~

~~Variable~~	~~Estimate~~	~~Default initial parameter values~~
~~lalpha~~	~~−4.00393~~	~~−0.0687608~~
~~rho~~	~~3.0323~~	~~0~~
~~intercept~~	~~4.16872~~	~~4.04~~
~~v~~	~~−1.74587~~	~~−1.55~~
~~n~~	~~1~~	~~2.12371~~
~~k~~	~~45.9212~~	~~74.4792~~

~~**Table of Data and Estimated Values of Interest**~~

~~Dose~~	~~N~~	~~Observed mean~~	~~Estimated mean~~	~~Observed SD~~	~~Estimated SD~~	~~Scaled residuals~~
~~0~~	~~8~~	~~4.04~~	~~4.17~~	~~1.42~~	~~1.18~~	~~−0.309~~
~~10.2~~	~~8~~	~~3.98~~	~~3.85~~	~~0.89~~	~~1.04~~	~~0.349~~
~~101~~	~~8~~	~~2.97~~	~~2.97~~	~~0.76~~	~~0.7~~	~~0.0059~~
~~1,008~~	~~8~~	~~2.49~~	~~2.5~~	~~0.59~~	~~0.54~~	~~−0.0466~~

~~**Likelihoods of Interest**~~

~~Model~~	~~Log (likelihood)~~	~~Number of parameters~~	~~AIC~~
~~A1~~	~~−12.763326~~	~~5~~	~~35.526651~~
~~A2~~	~~−9.319925~~	~~8~~	~~34.639851~~
~~A3~~	~~−9.91228~~	~~6~~	~~31.82456~~
~~fitted~~	~~−9.914356~~	~~5~~	~~29.828712~~
~~R~~	~~−19.643171~~	~~2~~	~~43.286341~~

~~**Tests of Interest**~~

~~Test~~	~~−2*log (likelihood ratio)~~	~~Test df~~	~~p-value~~
~~Test 1~~	~~20.6465~~	~~6~~	~~0.002123~~
~~Test 2~~	~~6.8868~~	~~3~~	~~0.07559~~

ED_005297A_00166423-00083

~~Test 3~~	~~1.18471~~	~~2~~	~~0.553~~
~~Test 4~~	~~0.00415236~~	~~1~~	~~0.9486~~

Exponential 4 Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is:

Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]

A modeled variance is fit

Benchmark Dose Computation

BMR = 15% RD

BMD = 39.1317

BMDL at the 95% confidence level = 11.5235

Parameter Estimates

Variable	Estimate	Default initial parameter values
lalpha	-3.94284	-3.54227
rho	2.98463	2.72754
a	4.1075	4.242
b	0.0123219	0.00282274
c	0.607906	0.55903
d	1 (specified)	1 (specified)

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	4.04	4.11	1.42	1.15	-0.167
10.2	8	3.98	3.92	0.89	1.07	0.166
101	8	2.97	2.961	0.76	0.71	0.036
1,008	8	2.49	2.50	0.59	0.55	-0.036

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-12.76333	5	35.52665
A2	-9.319925	8	34.63985
A3	-9.91228	6	31.82456
fitted	-9.966286	5	29.93257
R	-19.64317	2	43.28634

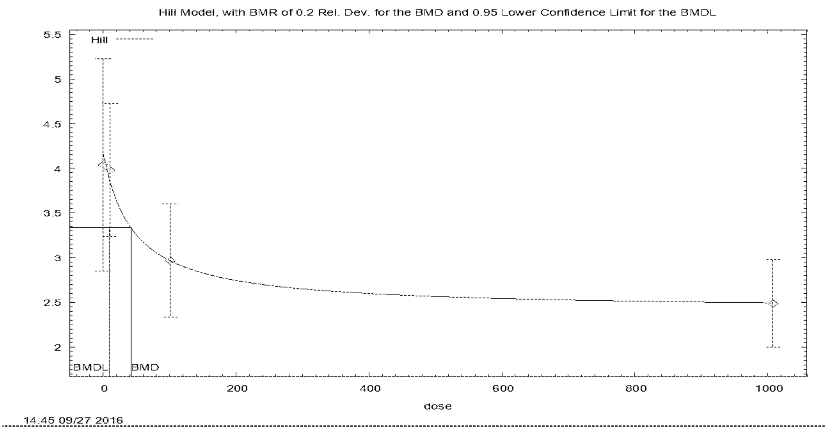
ED_005297A_00166423-00084

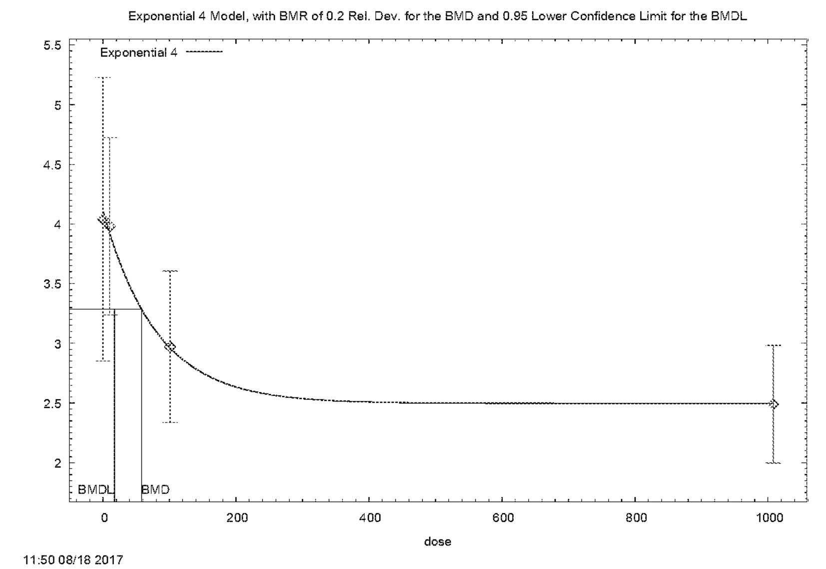
Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	20.65	6	0.002123
Test 2	6.887	3	0.07559
Test 3	1.185	2	0.553
Test 6a	0.108	1	0.7424

df = degree(s) of freedom

ED_005297A_00166423-00085





BMR = 20% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for Exponential 4 ~~Hill~~ Model, for T4 in F0 parental CRL Sprague-Dawley male rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential 4 Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is:

Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]

ED_005297A_00166423-00086

A modeled variance is fit

Benchmark Dose Computation

BMR = 20% RD

BMD = 57.9065

BMDL at the 95% confidence level = 17.1892

Parameter Estimates

Variable	Estimate	Default initial parameter values
lalpha	-3.94284	-3.54227
rho	2.98463	2.72754
a	4.1075	4.242
b	0.0123219	0.00282274
c	0.607906	0.55903
d	1 (specified)	1 (specified)

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	4.04	4.11	1.42	1.15	-0.167
10.2	8	3.98	3.92	0.89	1.07	0.166
101	8	2.97	2.961	0.76	0.71	0.036
1,008	8	2.49	2.50	0.59	0.55	-0.036

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-12.76333	5	35.52665
A2	-9.319925	8	34.63985
A3	-9.91228	6	31.82456
fitted	-9.966286	5	29.93257
R	-19.64317	2	43.28634

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	20.65	6	0.002123
Test 2	6.887	3	0.07559
Test 3	1.185	2	0.553
Test 6a	0.108	1	0.7424

~~df = degree(s) of freedom~~

~~**Hill Model** (Version: 2.17; Date: 01/28/2013)~~
~~The form of the response function is: Y[dose] = intercept + v*dose^n/(k^n + dose^n)~~
~~A modeled variance is fit~~

~~**Benchmark Dose Computation**~~
~~BMR = 20% RD~~
~~BMD = 41.9749~~
~~BMDL at the 95% confidence level = 9.10982~~

~~**Parameter Estimates**~~

~~Variable~~	~~Estimate~~	~~Default initial parameter values~~
~~lalpha~~	~~−4.00393~~	~~−0.0687608~~
~~rho~~	~~3.0323~~	~~0~~
~~intercept~~	~~4.16872~~	~~4.04~~
~~v~~	~~−1.74587~~	~~−1.55~~
~~n~~	~~1~~	~~2.12371~~
~~k~~	~~45.9212~~	~~74.4792~~

~~**Table of Data and Estimated Values of Interest**~~

~~Dose~~	~~N~~	~~Observed mean~~	~~Estimated mean~~	~~Observed SD~~	~~Estimated SD~~	~~Scaled residuals~~
~~0~~	~~8~~	~~4.04~~	~~4.17~~	~~1.42~~	~~1.18~~	~~−0.309~~
~~10.2~~	~~8~~	~~3.98~~	~~3.85~~	~~0.89~~	~~1.04~~	~~0.349~~
~~101~~	~~8~~	~~2.97~~	~~2.97~~	~~0.76~~	~~0.7~~	~~0.0059~~
~~1,008~~	~~8~~	~~2.49~~	~~2.5~~	~~0.59~~	~~0.54~~	~~−0.0466~~

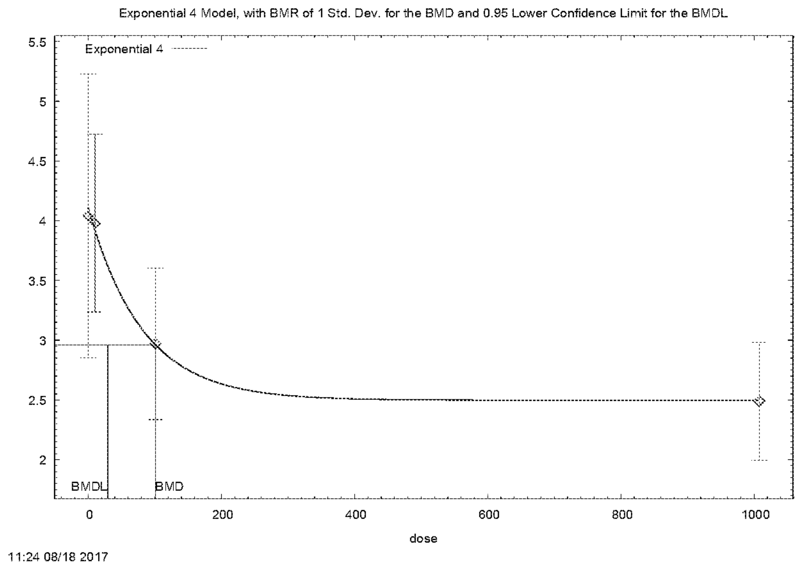
~~**Likelihoods of Interest**~~

~~Model~~	~~Log (likelihood)~~	~~Number of parameters~~	~~AIC~~
~~A1~~	~~−12.763326~~	~~5~~	~~35.526651~~
~~A2~~	~~−9.319925~~	~~8~~	~~34.639851~~
~~A3~~	~~−9.91228~~	~~6~~	~~31.82456~~
~~fitted~~	~~−9.914356~~	~~5~~	~~29.828712~~
~~R~~	~~−19.643171~~	~~2~~	~~43.286341~~

~~**Tests of Interest**~~

~~Test~~	~~−2*log (likelihood ratio)~~	~~Test df~~	~~*p*-value~~

~~Test 1~~	~~20.6465~~	~~6~~	~~0.002123~~
~~Test 2~~	~~6.8868~~	~~3~~	~~0.07559~~
~~Test 3~~	~~1.18471~~	~~2~~	~~0.553~~
~~Test 4~~	~~0.00415236~~	~~1~~	~~0.9486~~



BMR = 1 SD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for Exponential 4 Model, for T4 in F0 parental CRL Sprague-Dawley male rats exposed to HBCD by diet for 18 weeks (Ema, 2008, 787657).

Exponential 4 Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is:

Model 4: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A modeled variance is fit

Benchmark Dose Computation

BMR = 1 SD

BMD = 101.035

BMDL at the 95% confidence level = 29.4693

Parameter Estimates

Variable	Estimate	Default initial parameter values

ED_005297A_00166423-00089

lalpha	-3.94284	-3.54227
rho	2.98463	2.72754
a	4.1075	4.242
b	0.0123219	0.00282274
c	0.607906	0.55903
d	1 (specified)	1 (specified)

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	4.04	4.11	1.42	1.15	-0.167
10.2	8	3.98	3.92	0.89	1.07	0.166
101	8	2.97	2.961	0.76	0.71	0.036
1,008	8	2.49	2.50	0.59	0.55	-0.036

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-12.76333	5	35.52665
A2	-9.319925	8	34.63985
A3	-9.91228	6	31.82456
fitted	-9.966286	5	29.93257
R	-19.64317	2	43.28634

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	20.65	6	0.002123
Test 2	6.887	3	0.07559
Test 3	1.185	2	0.553
Test 6a	0.108	1	0.7424

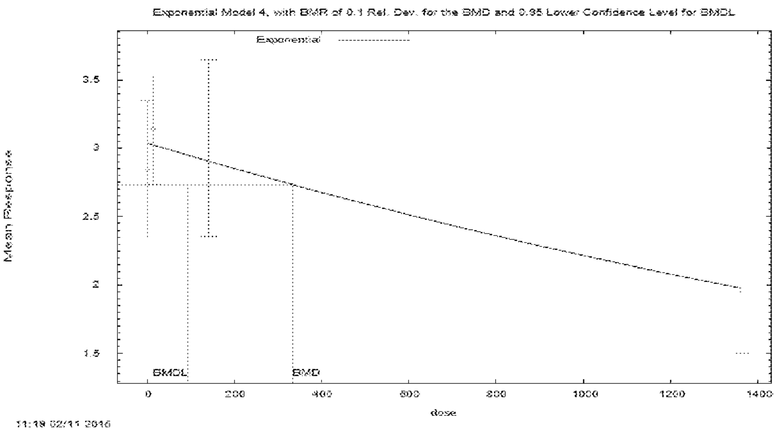
df = degree(s) of freedom

Table D-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for T4 in F0 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}; BMR = 10% RD from control mean, 15% RD from control mean, 20% RD from control mean, and 1 SD change from control mean

Model[a]	Goodness of fit *p*-value	Goodness of fit AIC	BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{15RD} (mg/kg-d)	$BMDL_{15RD}$ (mg/kg-d)	Basis for model selection
Exponential (M2)	0.479	3.7677	334	225	516	348	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential M4 constant variance model was selected based on lowest BMDL (BMDLs differed by >3).
Exponential (M3)	0.298	5.3774	1,065	232	1,150	357	
Exponential (M4)	**0.479**	**3.7677**	**334**	**93.8**	**516**	**154**	
Exponential (M5)	N/A[b]	7.3774	1,086	103	1,158	143	
Hill	N/A[b]	7.3774	1,067	100	1,138	error[c]	
Power	0.298	5.3774	1,171	293	1,230	439	
Polynomial 3°	0.582	3.3778	902	816	1,032	934	
Polynomial 2°	0.580	3.3836	733	293	897	439	
Linear	0.505	3.6625	389	289	584	433	
Model[a]	**Goodness of fit *p*-value**	**Goodness of fit AIC**	**BMD_{20RD} (mg/kg-d)**	**$BMDL_{20RD}$ (mg/kg-d)**	**BMD_{1SD} (mg/kg-d)**	**$BMDL_{1SD}$ (mg/kg-d)**	
Exponential (M2)	0.479	3.7677	708	477	680	433	
Exponential (M3)	0.298	5.3774	1,240	491	1,234	446	
Exponential (M4)	**0.479**	**3.7677**	**708**	**229**	**680**	**211**	
Exponential (M5)	N/A[b]	7.3774	1,217	146	1,211	145	
Hill	N/A[b]	7.3774	1,185	error[c]	1,178	error[c]	
Power	0.298	5.3774	1,275	586	1,270	532	
Polynomial 3°	0.582	3.3778	1,136	1,028	1,126	999	
Polynomial 2°	0.580	3.3836	1,036	586	1,021	532	
Linear	0.505	3.6625	779	577	751	523	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.579), selected model in bold; scaled residuals for selected model for doses 0, 14, 141.3, and 1,363 mg/kg-day were −0.9501, 0.5631, 0.4611, and −0.07911, respectively.
[b]No available degrees of freedom to calculate a goodness-of-fit value.
[c]BMD or BMDL computation failed for this model.

ED_005297A_00166423-00091



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for Exponential Model 4, for T4 in F0 parental CRL Sprague-Dawley female rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.9; Date: 01/29/2013)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 334.313

BMDL at the 95% confidence level = 93.781

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−1.06976	−1.11576
rho(S)	N/A	0
a	3.03677	3.297
b	0.000315155	0.00199958
c	0	0.566171
d	1	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	2.84	3.037	0.61	0.5857	−0.9501
14	8	3.14	3.023	0.48	0.5857	0.5631
141.3	8	3	2.905	0.77	0.5857	0.4611
1,363	8	1.96	1.976	0.55	0.5857	−0.07911

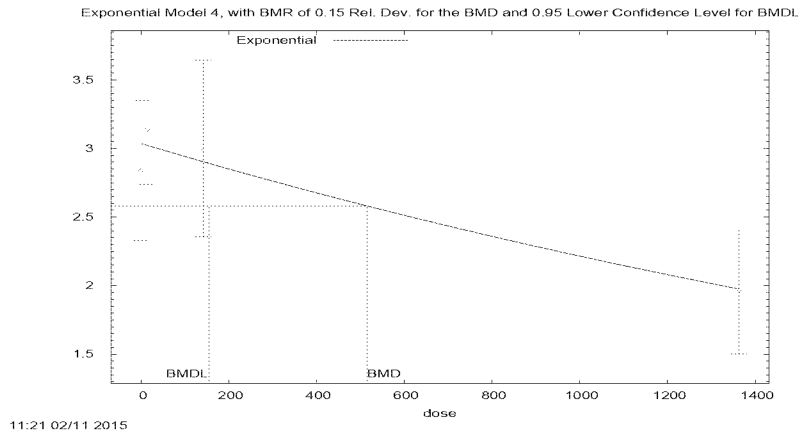
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	1.852186	5	6.295628
A2	2.83624	8	10.32752
A3	1.852186	5	6.295628
R	−6.115539	2	16.23108
4	1.116152	3	3.767695

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	17.9	6	0.006478
Test 2	1.968	3	0.5791
Test 3	1.968	3	0.5791
Test 6a	1.472	2	0.479

ED_005297A_00166423-00093



BMR = 15% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for Exponential Model 4, for T4 in F0 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.9; Date: 01/29/2013)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 15% RD

BMD = 515.679

BMDL at the 95% confidence level = 154.19

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−1.06976	−1.11576
rho(S)	N/A	0
a	3.03677	3.297
b	0.000315155	0.00199958
c	0	0.566171
d	1	1

Table of Data and Estimated Values of Interest

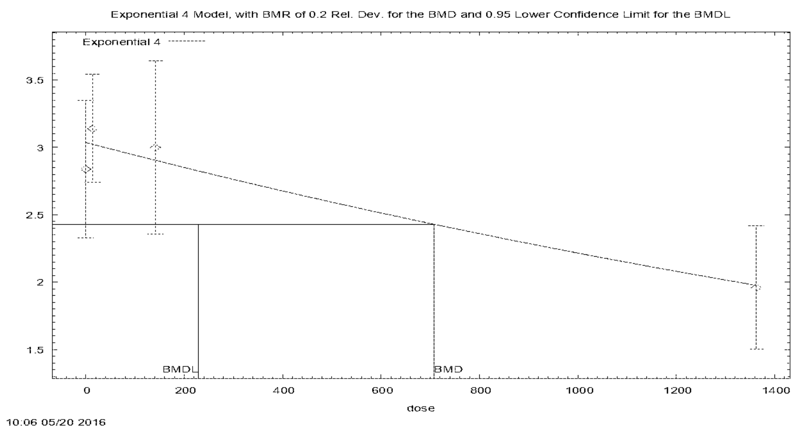
Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	2.84	3.037	0.61	0.5857	−0.9501
14	8	3.14	3.023	0.48	0.5857	0.5631
141.3	8	3	2.905	0.77	0.5857	0.4611
1,363	8	1.96	1.976	0.55	0.5857	−0.07911

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	1.852186	5	6.295628
A2	2.83624	8	10.32752
A3	1.852186	5	6.295628
R	−6.115539	2	16.23108
4	1.116152	3	3.767695

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	17.9	6	0.006478
Test 2	1.968	3	0.5791
Test 3	1.968	3	0.5791
Test 6a	1.472	2	0.479



BMR = 20% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for T4 in F0 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 20% RD

BMD = 708.043

BMDL at the 95% confidence level = 228.829

Parameter Estimates

Variable	Estimate	Default initial parameter values
Lnalpha	−1.06976	−1.11576
Rho	N/A	0
A	3.03677	3.297
B	0.000315155	0.00199958
C	0	0.566171
D	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	2.84	3.04	0.61	0.59	−0.9501
14	8	3.14	3.02	0.48	0.59	0.5631
141.3	8	3	2.9	0.77	0.59	0.4611
1,363	8	1.96	1.98	0.55	0.59	−0.07911

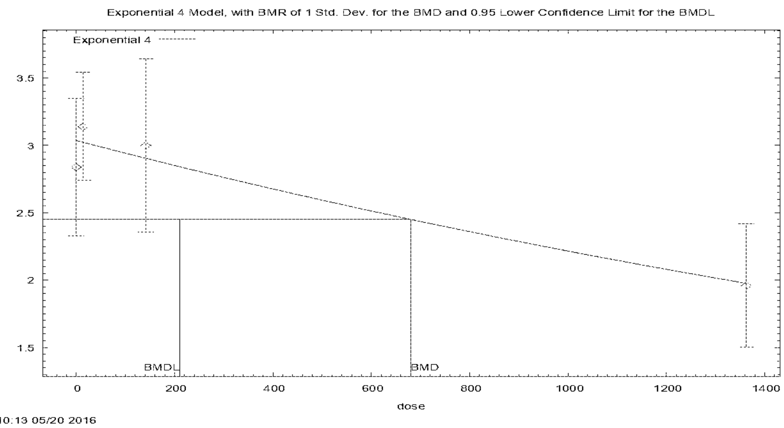
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	1.852186	5	6.295628
A2	2.83624	8	10.32752
A3	1.852186	5	6.295628
R	−6.115539	2	16.23108
4	1.116152	3	3.767695

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	17.9	6	0.006478
Test 2	1.968	3	0.5791
Test 3	1.968	3	0.5791
Test 6a	1.472	2	0.479

ED_005297A_00166423-00097



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for T4 in F0 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 1.0000 Estimated SDs from control

BMD = 679.939

BMDL at the 95% confidence level = 210.769

Parameter Estimates

Variable	Estimate	Default initial parameter values
Lnalpha	−1.06976	−1.11576
Rho	N/A	0
A	3.03677	3.297
B	0.000315155	0.00199958
C	0	0.566171
D	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	2.84	3.04	0.61	0.59	−0.9501
14	8	3.14	3.02	0.48	0.59	0.5631
141.3	8	3	2.9	0.77	0.59	0.4611
1,363	8	1.96	1.98	0.55	0.59	−0.07911

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	1.852186	5	6.295628
A2	2.83624	8	10.32752
A3	1.852186	5	6.295628
R	−6.115539	2	16.23108
4	1.116152	3	3.767695

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	17.9	6	0.006478
Test 2	1.968	3	0.5791
Test 3	1.968	3	0.5791
Test 6a	1.472	2	0.479

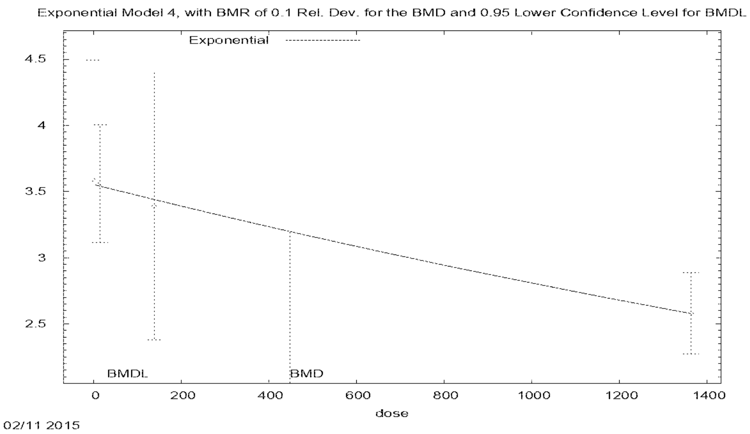
ED_005297A_00166423-00099

Table D-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for T4 in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}; BMR = 10% RD from control mean, 15% RD from control mean, 20% RD from control mean, and 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{15RD} (mg/kg-d)	$BMDL_{15RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2)	0.305	19.978	448	320	691	493	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential M4 (modeled variance) model was selected based on lowest BMDL (BMDLs differed by >3).
Exponential (M3)	0.191	21.318	1,184	333	1,254	514	
Exponential (M4)	**0.305**	**19.978**	**448**	**127**	**691**	**214**	
Exponential (M5)	N/A[b]	23.318	1,193	153	1,259	144	
Hill	N/A[b]	23.318	1,131	153	1,204	error[c]	
Power	0.191	21.318	1,287	389	1,318	583	
Polynomial 3°	0.424	19.323	984	898	1,127	1,028	
Polynomial 2°	0.414	19.368	835	728	1,023	892	
Linear	0.323	19.868	498	379	747	568	
Model[a]	**Goodness of fit**		**BMD_{20RD} (mg/kg-d)**	**$BMDL_{20RD}$ (mg/kg-d)**	**BMD_{1SD} (mg/kg-d)**	**$BMDL_{1SD}$ (mg/kg-d)**	
	***p*-value**	**AIC**					
Exponential (M2)	0.305	19.978	948	677	1,344	828	
Exponential (M3)	0.191	21.318	1,305	705	1,362	876	
Exponential (M4)	**0.305**	**19.978**	**948**	**328**	**1,344**	**536**	
Exponential (M5)	N/A[b]	23.318	1,309	148	1,362	152	
Hill	N/A[b]	23.318	1,269	error[c]	1,360	error[c]	
Power	0.191	21.318	1,341	777	1,363	932	
Polynomial 3°	0.424	19.323	1,240	1,132	1,360	1,193	
Polynomial 2°	0.414	19.368	1,181	1,030	1,357	1,115	
Linear	0.323	19.868	996	757	1,344	896	

[a]Modeled variance case presented (BMDS Test 2 *p*-value = 0.00445), selected model in bold; scaled residuals for selected model for doses 0, 14.3, 138.3, and 1,363 mg/kg-day were 0.105, 0.05257, −0.1637, and 0.008804, respectively.
[b]No available degrees of freedom to calculate a goodness-of-fit value.
[c]BMD or BMDL computation failed for this model.

ED_005297A_00166423-00100



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for Exponential Model 4 (modeled variance) for T4 in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.9; Date: 01/29/2013)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A modeled variance is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 447.782

BMDL at the 95% confidence level = 127.272

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−7.9144	−6.73265
rho	6.1823	5.13248
a	3.55422	3.7695
b	0.000235294	0.000283737
c	0	0.000684441
d	1	1

ED_005297A_00166423-00101

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	3.59	3.554	1.08	0.9635	0.105
14.3	8	3.56	3.542	0.53	0.9535	0.05257
138.3	8	3.39	3.44	1.21	0.8713	−0.1637
1,363	8	2.58	2.579	0.37	0.3574	0.008804

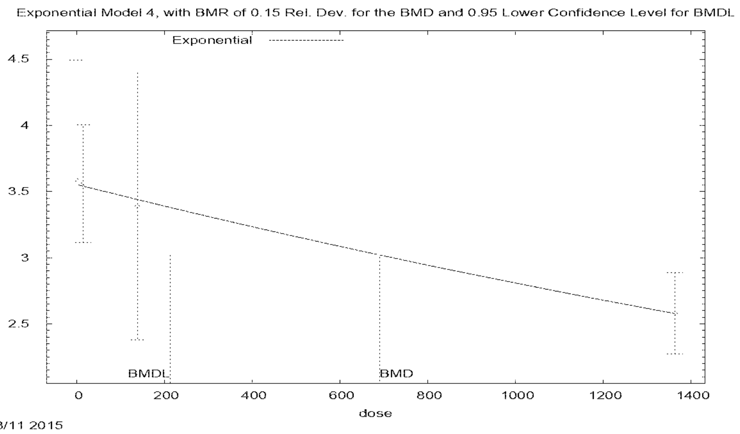
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−9.516133	5	29.03227
A2	−2.971105	8	21.94221
A3	−4.802103	6	21.60421
R	−13.13332	2	30.26663
4	−5.988946	4	19.97789

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	20.32	6	0.002424
Test 2	13.09	3	0.004446
Test 3	3.662	2	0.1603
Test 6a	2.374	2	0.3052

ED_005297A_00166423-00102



BMR = 15% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for Exponential Model 4, for T4 in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.9; Date: 01/29/2013)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A modeled variance is fit

Benchmark Dose Computation

BMR = 15% RD

BMD = 690.705

BMDL at the 95% confidence level = 213.844

Parameter Estimates

Variable	Estimate	Default initial parameter values
Lnalpha	−7.9144	−6.73265
Rho	6.1823	5.13248
A	3.55422	3.7695
B	0.000235294	0.000283737
C	0	0.000684441
D	1	1

ED_005297A_00166423-00103

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	3.59	3.554	1.08	0.9635	0.105
14.3	8	3.56	3.542	0.53	0.9535	0.05257
138.3	8	3.39	3.44	1.21	0.8713	−0.1637
1,363	8	2.58	2.579	0.37	0.3574	0.008804

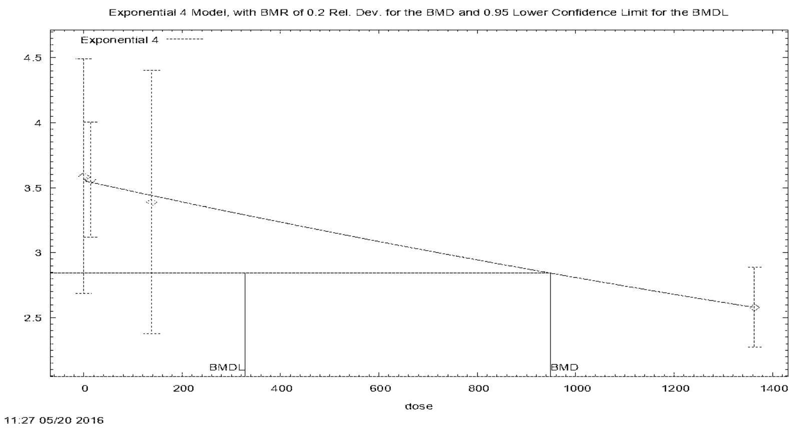
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−9.516133	5	29.03227
A2	−2.971105	8	21.94221
A3	−4.802103	6	21.60421
R	−13.13332	2	30.26663
4	−5.988946	4	19.97789

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	20.32	6	0.002424
Test 2	13.09	3	0.004446
Test 3	3.662	2	0.1603
Test 6a	2.374	2	0.3052

ED_005297A_00166423-00104



BMR = 20% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with modeled variance for T4 in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A modeled variance is fit

Benchmark Dose Computation

BMR = 20% RD

BMD = 948.359

BMDL at the 95% confidence level = 328.063

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−7.9144	−6.73265
rho	6.1823	5.13248
a	3.55422	3.7695
b	0.000235294	0.000283737
c	0	0.000684441
d	N/A	1

Table of Data and Estimated Values of Interest

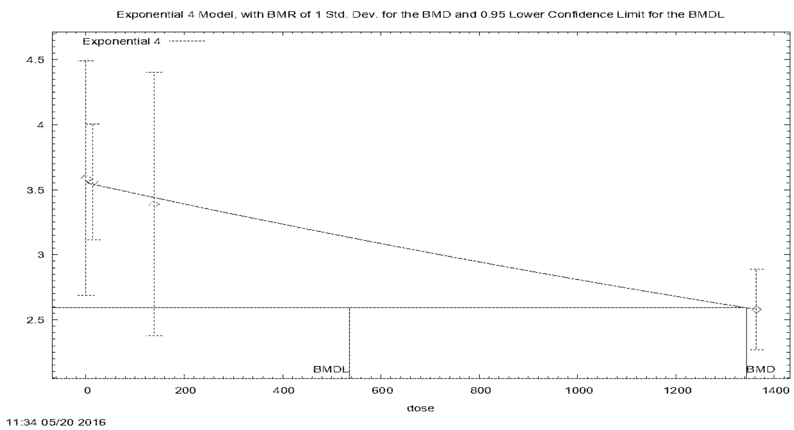
Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	3.59	3.55	1.08	0.96	0.105
14.3	8	3.56	3.54	0.53	0.95	0.05257
138.3	8	3.39	3.44	1.21	0.87	−0.1637
1,363	8	2.58	2.58	0.37	0.36	0.008804

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−9.516133	5	29.03227
A2	−2.971105	8	21.94221
A3	−4.802103	6	21.60421
R	−13.13332	2	30.26663
4	−5.988946	4	19.97789

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	20.32	6	0.002424
Test 2	13.09	3	0.004446
Test 3	3.662	2	0.1603
Test 6a	2.374	2	0.3052



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with modeled variance for T4 in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A modeled variance is fit

Benchmark Dose Computation

BMR = 1.0000 Estimated SDs from control

BMD = 1,343.81

BMDL at the 95% confidence level = 536.006

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−7.9144	−6.73265
rho	6.1823	5.13248
a	3.55422	3.7695
b	0.000235294	0.000283737
c	0	0.000684441
d	N/A	1

ED_005297A_00166423-00107

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	3.59	3.55	1.08	0.96	0.105
14.3	8	3.56	3.54	0.53	0.95	0.05257
138.3	8	3.39	3.44	1.21	0.87	−0.1637
1,363	8	2.58	2.58	0.37	0.36	0.008804

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−9.516133	5	29.03227
A2	−2.971105	8	21.94221
A3	−4.802103	6	21.60421
R	−13.13332	2	30.26663
4	−5.988946	4	19.97789

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	20.32	6	0.002424
Test 2	13.09	3	0.004446
Test 3	3.662	2	0.1603
Test 6a	2.374	2	0.3052

ED_005297A_00166423-00108

D.2.3.2 *Liver*

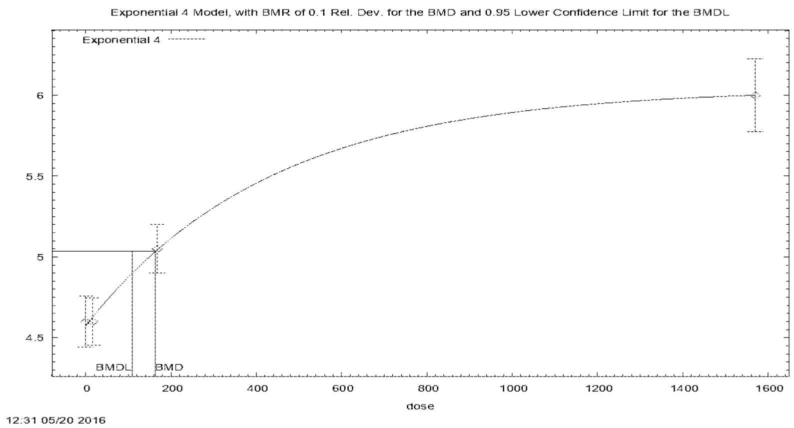
Table D-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for relative liver weight (g/100 g BW) in male F1 CRL rats exposed to HBCD on GD 0–PND 26, dose TWA gestation through lactation {Ema, 2008, 787657}; BMR = 10% RD from control mean and 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2) Exponential (M3)[b]	0.00369	−70.405	599	533	488	417	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential M4 constant variance model was selected based on lowest AIC and visual fit.
Exponential (M4)	**0.606**	**−79.345**	**163**	**109**	**120**	**80.5**	
Exponential (M5)	N/A[c]	−77.611	169	111	157	82.0	
Hill	N/A[c]	−77.611	169	104	156	75.4	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.00590	−71.344	548	480	440	371	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.462), selected model in bold; scaled residuals for selected model for doses 0, 16.5, 168, and 1,570 mg/kg-day were 0.3267, −0.3947, 0.05759, and −0.003788, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]No available degrees of freedom to calculate a goodness-of-fit value.
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e]For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from {Ema, 2008, 787657@@author-year}

ED_005297A_00166423-00109



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F1 weanling male CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA gestation through lactation {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 162.81

BMDL at the 95% confidence level = 108.569

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−2.07833	−2.08162
rho	N/A	0
a	4.5759	4.37
b	0.00230233	0.00120199
c	1.3199	1.44165
d	N/A	1

ED_005297A_00166423-00110

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	23	4.6	4.576	0.37	0.3538	0.3267
16.5	21	4.6	4.63	0.32	0.3538	−0.3947
168	20	5.05	5.045	0.32	0.3538	0.05759
1,570	17	6	6	0.44	0.3538	−0.003788

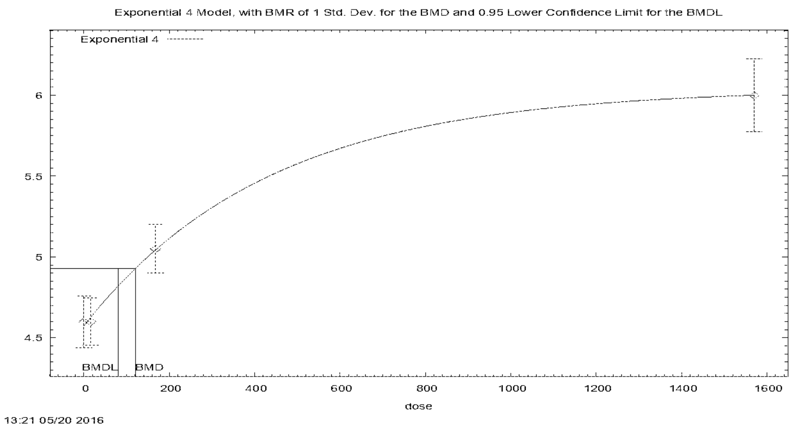
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	43.80548	5	−77.61096
A2	45.09301	8	−74.18602
A3	43.80548	5	−77.61096
R	−5.569318	2	15.13864
4	43.67234	4	−79.34469

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	101.3	6	<0.0001
Test 2	2.575	3	0.4619
Test 3	2.575	3	0.4619
Test 6a	0.2663	1	0.6058

ED_005297A_00166423-00111



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F1 weanling male CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA gestation through lactation {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 1.0000 Estimated SDs from control

BMD = 120.152

BMDL at the 95% confidence level = 80.5016

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−2.07833	−2.08162
rho	N/A	0
a	4.5759	4.37
b	0.00230233	0.00120199
c	1.3199	1.44165
d	N/A	1

ED_005297A_00166423-00112

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	23	4.6	4.576	0.37	0.3538	0.3267
16.5	21	4.6	4.63	0.32	0.3538	−0.3947
168	20	5.05	5.045	0.32	0.3538	0.05759
1,570	17	6	6	0.44	0.3538	−0.003788

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	43.80548	5	−77.61096
A2	45.09301	8	−74.18602
A3	43.80548	5	−77.61096
R	−5.569318	2	15.13864
4	43.67234	4	−79.34469

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	101.3	6	<0.0001
Test 2	2.575	3	0.4619
Test 3	2.575	3	0.4619
Test 6a	0.2663	1	0.6058

Table D-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for relative liver weight (g/100 g BW) in F1 weanling female CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA of gestation and lactation {Ema, 2008, 787657}; BMR = 10% RD from control mean and 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2) Exponential (M3)[b]	0.00217	−82.410	560	503	418	359	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential M4 constant variance model was selected based on lowest AIC.
Exponential (M4)	**0.731**	**−92.555**	**165**	**115**	**109**	**75.8**	
Exponential (M5)	N/A[c]	−90.673	170	116	126	76.4	
Hill	N/A[c]	−90.673	170	110	124	70.8	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear[g]	0.00403	−83.646	507	449	371	315	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.711), selected model in bold; scaled residuals for selected model for doses 0, 16.5, 168, and 1,570 mg/kg-day were 0.2185, −0.263, 0.03719, and −0.002332, respectively.

[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

[c]No available degrees of freedom to calculate a goodness-of-fit value.

[d]The Power model may appear equivalent to the Linear model; however, differences exist in digits not displayed in the table.

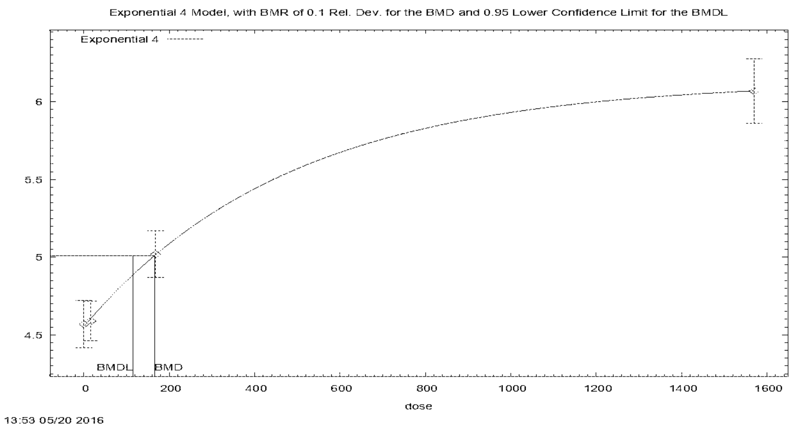
[e]For the Polynomial 3° model, the b3 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 2° model.

[f]The Polynomial 2° model may appear equivalent to the Linear model; however, differences exist in digits not displayed in the table.

[g]The Linear model may appear equivalent to the Power model; however, differences exist in digits not displayed in the table. This also applies to the Polynomial 3° and Polynomial 2° models.

Data from {Ema, 2008, 787657@@author-year}

ED_005297A_00166423-00114



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F1 weanling female CRL Sprague-Dawley rats exposed to HBCD GD 0–PND 26, dose TWA of gestation and lactation {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 165.267

BMDL at the 95% confidence level = 114.71

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−2.28916	−2.29068
rho	N/A	0
a	4.5555	4.3415
b	0.00206359	0.00122548
c	1.34605	1.46804
d	N/A	1

ED_005297A_00166423-00115

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	23	4.57	4.555	0.35	0.3184	0.2185
16.5	21	4.59	4.608	0.28	0.3184	−0.263
168	20	5.02	5.017	0.32	0.3184	0.03719
1,570	14	6.07	6.07	0.36	0.3184	−0.002332

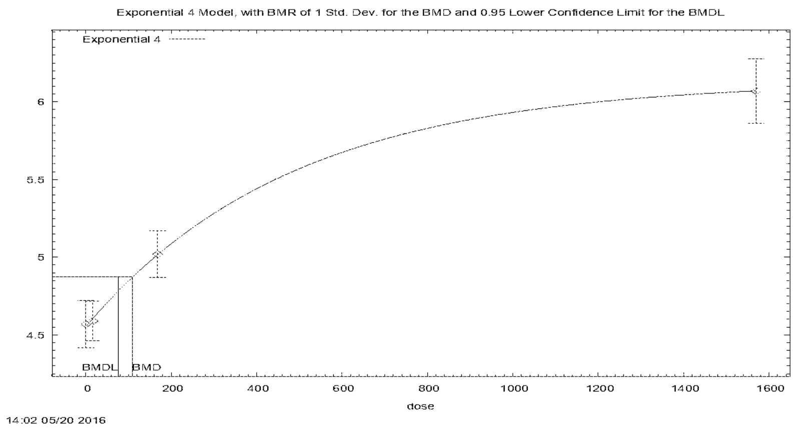
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	50.33659	5	−90.67319
A2	51.02517	8	−86.05034
A3	50.33659	5	−90.67319
R	−3.746671	2	11.49334
4	50.2774	4	−92.55481

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	109.5	6	<0.0001
Test 2	1.377	3	0.7109
Test 3	1.377	3	0.7109
Test 6a	0.1184	1	0.7308

ED_005297A_00166423-00116



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F1 weanling female CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA of gestation and lactation {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 1.0000 Estimated SDs from control

BMD = 109.314

BMDL at the 95% confidence level = 75.8445

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−2.28916	−2.29068
rho	N/A	0
a	4.5555	4.3415
b	0.00206359	0.00122548
c	1.34605	1.46804
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	23	4.57	4.555	0.35	0.3184	0.2185
16.5	21	4.59	4.608	0.28	0.3184	−0.263
168	20	5.02	5.017	0.32	0.3184	0.03719
1,570	14	6.07	6.07	0.36	0.3184	−0.002332

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	50.33659	5	−90.67319
A2	51.02517	8	−86.05034
A3	50.33659	5	−90.67319
R	−3.746671	2	11.49334
4	50.2774	4	−92.55481

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	109.5	6	<0.0001
Test 2	1.377	3	0.7109
Test 3	1.377	3	0.7109
Test 6a	0.1184	1	0.7308

ED_005297A_00166423-00118

Table D-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for relative liver weight (g/100 g BW) in F1 adult male CRL Sprague-Dawley rats exposed to HBCD by diet for 15 weeks {Ema, 2008, 787657}; BMR = 10% RD from control mean and 1 SD change from control mean.

Model[a]	Goodness of fit *p*-value	Goodness of fit AIC	BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	Basis for model selection
Exponential (M2) Exponential (M3)[b]	0.626	−167.34	703	601	519	433	Of the models that provided an adequate fit and a valid BMDL estimate, the ~~Exponential M4~~Linear constant variance model was selected based on lowest AIC~~BMDL~~ (BMDLs differed by <~~>~~3). Exponential M5 and Hill models wwere~~as~~ excluded because ~~it has four dose groups~~both were saturated models in this case. ~~; the model fit is more likely to be biased by the form of the model, which can result in a misrepresentation of the true dose-response shape.~~
Exponential (M4)	0.366	−165.46	578	243	402	161	
Exponential (M5)	0.366	−165.46	578	121	402	118	
Hill	0.367	−165.46	582	error[c]	404	164	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	**0.638**	**−167.38**	**680**	**573**	**496**	**409**	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.181), selected model in bold; scaled residuals for selected model for doses 0, 11.4, 115, and 1,142 mg/kg-day were -0.723~~−0.596~~, 0.587~~0.6713~~, 0.165~~−0.07974~~, and -0.0218~~0.001037~~, respectively.

[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

[c]BMD or BMDL computation failed for this model.

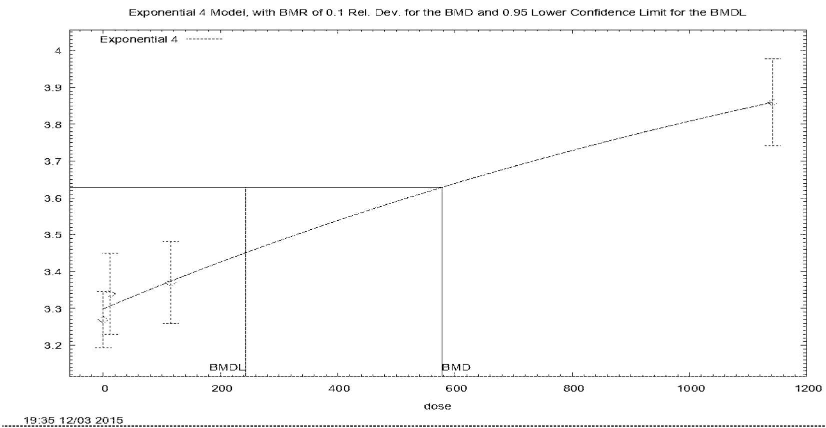
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.

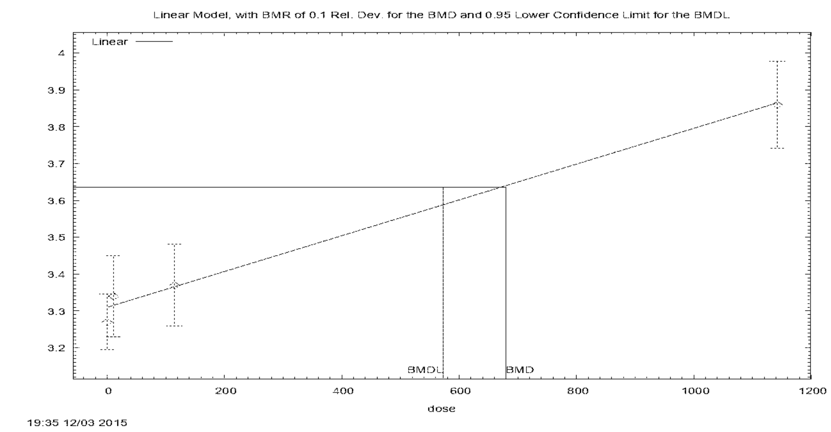
[e]For the Polynomial 3° model, the b3 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.

[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from {Ema, 2008, 787657@@author-year}

ED_005297A_00166423-00119





BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for ~~Exponential (M4)~~Linear model with constant variance for relative liver weight (g/100 g BW) in F1 adult male CRL Sprague-Dawley rats exposed to HBCD by diet for 15 weeks {Ema, 2008, 787657}.

~~**Exponential Model** (Version: 1.10; Date: 01/12/2015)~~

~~The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]~~

~~A constant variance model is fit~~

~~**Benchmark Dose Computation**~~

~~BMR = 10% RD~~

~~BMD = 578.114~~

ED_005297A_00166423-00120

~~BMDL at the 95% confidence level = 242.728~~

~~**Parameter Estimates**~~

~~Variable~~	~~Estimate~~	~~Default initial parameter values~~
~~lnalpha~~	~~−2.84531~~	~~−2.85399~~
~~rho~~	~~N/A~~	~~0~~
~~a~~	~~3.29933~~	~~3.1065~~
~~b~~	~~0.000582616~~	~~0.00140918~~
~~c~~	~~1.3497~~	~~1.30468~~
~~d~~	~~N/A~~	~~1~~

~~**Table of Data and Estimated Values of Interest**~~

~~Dose~~	~~N~~	~~Observed mean~~	~~Estimated mean~~	~~Observed SD~~	~~Estimated SD~~	~~Scaled residuals~~
~~0~~	~~24~~	~~3.27~~	~~3.299~~	~~0.18~~	~~0.2411~~	~~−0.596~~
~~11.4~~	~~24~~	~~3.34~~	~~3.307~~	~~0.26~~	~~0.2411~~	~~0.6713~~
~~115~~	~~22~~	~~3.37~~	~~3.374~~	~~0.25~~	~~0.2411~~	~~−0.07974~~
~~1,142~~	~~24~~	~~3.86~~	~~3.86~~	~~0.28~~	~~0.2411~~	~~0.001037~~

~~**Likelihoods of Interest**~~

~~Model~~	~~Log (likelihood)~~	~~Number of parameters~~	~~AIC~~
~~A1~~	~~87.13765~~	~~5~~	~~−164.2753~~
~~A2~~	~~89.57845~~	~~8~~	~~−163.1569~~
~~A3~~	~~87.13765~~	~~5~~	~~−164.2753~~
~~R~~	~~55.37316~~	~~2~~	~~−106.7463~~
~~4~~	~~86.72978~~	~~4~~	~~−165.4596~~

~~**Tests of Interest**~~

~~Test~~	~~−2*log (likelihood ratio)~~	~~Test df~~	~~*p*-value~~
~~Test 1~~	~~68.41~~	~~6~~	~~<0.0001~~
~~Test 2~~	~~4.882~~	~~3~~	~~0.1807~~
~~Test 3~~	~~4.882~~	~~3~~	~~0.1807~~
~~Test 6a~~	~~0.8158~~	~~1~~	~~0.3664~~

Polynomial Model. (Version: 2.20; Date: 10/22/2014)
The form of the response function is: Y[dose] = beta_0 + beta_1*dose
A constant variance model is fit

ED_005297A_00166423-00121

Benchmark Dose Computation.

BMR = 10% Relative deviation

BMD = 679.573

BMDL at the 95% confidence level = 572.977

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
alpha	0.0581671	0.0601744
rho	n/a	0
beta_0	3.30558	3.30581
beta_1	0.00048642	0.000486264

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Resid
0	24	3.27	3.31	0.18	0.241	-0.723
11.4	24	3.34	3.31	0.26	0.241	0.587
115	22	3.37	3.36	0.25	0.241	0.165
1142	24	3.86	3.86	0.28	0.241	-0.0218

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	87.137654	5	-164.275308
A2	89.578448	8	-163.156897
A3	87.137654	5	-164.275308
fitted	86.688502	3	-167.377004
R	55.373159	2	-106.746318

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	68.4106	6	<0.0001
Test 2	4.88159	3	0.1807
Test 3	4.88159	3	0.1807
Test 4	0.898304	2	0.6382

Table D-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for relative liver weight (g/100g bw) in F1 adult female CRL Sprague-Dawley rats exposed to HBCD by diet for 17 weeks {Ema, 2008, 787657}; BMR = 10% RD from control mean and 1 SD change from control mean

Model[a]	Goodness of fit *p*-value	Goodness of fit AIC	BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	Basis for model selection
Exponential (M2) Exponential (M3)[b]	0.311	−40.783	791	615	824	635	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential M4 constant variance model was selected based on lowest BMDL (BMDLs differed by >3). ~~Exponential M5~~Hill model was excluded because it was a saturated model in this case.~~has four dose groups; the model fit is more likely to be biased by the form of the model, which can result in a misrepresentation of the true dose-response shape.~~
Exponential (M4) Exponential (M5)[c]	**0.139**	**−38.934**	**569**	**184**	**603**	**203**	
Hill	0.139	−38.937	575	186	610	208	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear[g]	0.316	−40.816	761	578	795	598	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.917), selected model in bold; scaled residuals for selected model for doses 0, 14.3, 138, and 1,363 mg/kg-d were −0.9658, 1.098, −0.1406, and 0.002993, respectively.

[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

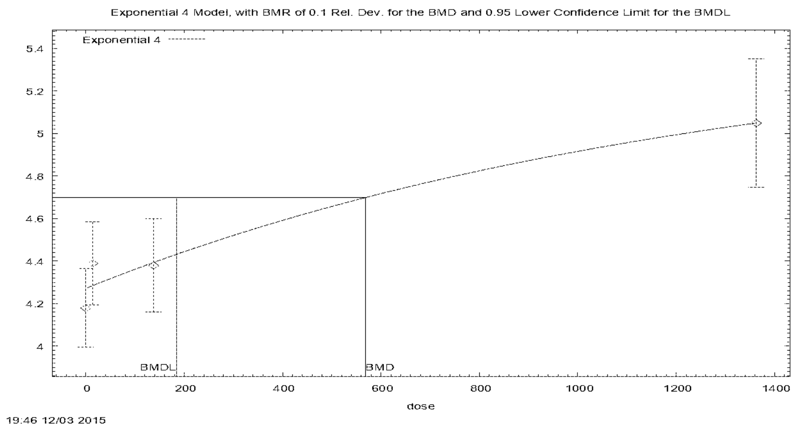
[c]The Exponential (M5) model may appear equivalent to the Exponential (M4) model; however, differences exist in digits not displayed in the table.

[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.

[e]For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.

[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from {Ema, 2008, 787657@@author-year}



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F1 adult female CRL Sprague-Dawley rats exposed to HBCD by diet for 17 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 568.784

BMDL at the 95% confidence level = 184.198

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−1.60953	−1.63795
rho	N/A	0
a	4.27208	3.971
b	0.000792725	0.0012372
c	1.27553	1.33531
d	N/A	1

ED_005297A_00166423-00124

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	4.18	4.272	0.42	0.4472	−0.9658
14.3	22	4.39	4.285	0.44	0.4472	1.098
138	20	4.38	4.394	0.47	0.4472	−0.1406
1,363	13	5.05	5.05	0.5	0.4472	0.002993

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	24.56111	5	−39.12222
A2	24.8146	8	−33.6292
A3	24.56111	5	−39.12222
R	10.7627	2	−17.5254
4	23.46704	4	−38.93407

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	28.1	6	<0.0001
Test 2	0.507	3	0.9174
Test 3	0.507	3	0.9174
Test 6a	2.188	1	0.1391

ED_005297A_00166423-00125

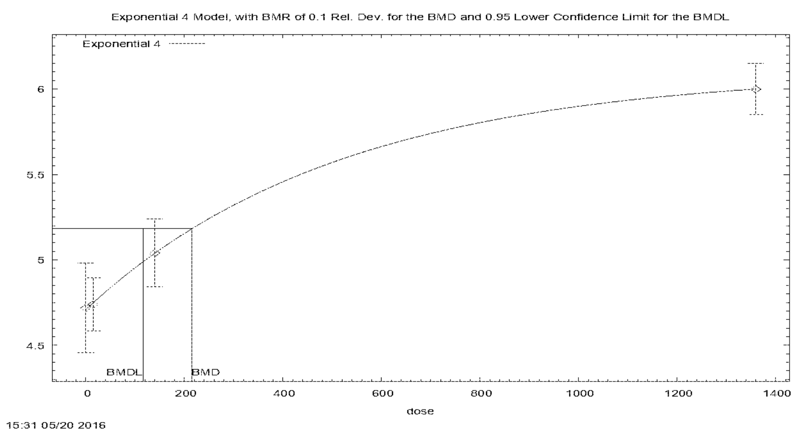
Table D-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for relative liver weight (g/100 g BW) in F2 weanling male CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA gestation and lactation {Ema, 2008, 787657}; BMR = 10% RD from control mean and 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2) Exponential (M3)[b]	0.235	−45.537	563	482	587	488	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential M4 constant variance model was selected based on lowest BMDL (BMDLs differed by >3).
Exponential (M4)	**0.882**	**−46.411**	**215**	**116**	**227**	**125**	
Exponential (M5)	N/A[c]	−44.433	200	116	218	125	
Hill	N/A[c]	−44.433	207	112	223	120	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.278	−45.874	522	438	540	441	

[a]Constant variance case presented. Both constant variance assumption and modeled variance were not appropriate in this case: BMDS Tests 2 and 3 with constatnt variance assumption rejected the null hypothesis with *p*-value = 0.00438; Test 3 of modeled variance also rejected the null hypothesis. A sensitivity analysis (see below) indicated limited effect of variance on model fitting. Selected model in bold; scaled residuals for selected model for doses 0, 14.7, 139.3, and 1,360 mg/kg-day were 0.09694, −0.1119, 0.01719, and −0.0007502, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]No available degrees of freedom to calculate a goodness-of-fit value.
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e]For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from {Ema, 2008, 787657@@author-year}

ED_005297A_00166423-00126



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F2 weanling male CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA gestation and lactation {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 214.961

BMDL at the 95% confidence level = 115.944

Parameter Estimates

Variable	Estimate	Default initial parameter values
Lnalpha	−1.72548	−1.72578
Rho	N/A	0
A	4.71128	4.484
B	0.00192508	0.00133871
C	1.29509	1.405
D	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	4.72	4.711	0.59	0.422	0.09694
14.7	22	4.74	4.75	0.35	0.422	−0.1119
139.3	18	5.04	5.038	0.4	0.422	0.01719
1,360	13	6	6	0.25	0.422	−0.0007502

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	27.21664	5	−44.43327
A2	33.77721	8	−51.55442
A3	27.21664	5	−44.43327
R	−2.570126	2	9.140253
4	27.20553	4	−46.41105

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	72.69	6	<0.0001
Test 2	13.12	3	0.004382
Test 3	13.12	3	0.004382
Test 6a	0.02222	1	0.8815

Sensitivity analysis:

The fit to the means was adequate for Exponential M4 with constant variance, and their scaled residuals were small. However, Tests 2 and 3 rejected the null hypothesis with both constant variance assumption and modeled variance, indicating lack of fit to variances whether the variance was constant or modeled as a power of the means. To determine how much $BMDL_{10\%RD}$ (116 mg/kg-day) was affected by the variance used, a sensitivity analysis was performed with constant variance by setting the standard deviation for all dose groups to the minimum or maximum observed values (0.25 and 0.59). Because the means were not changed and the constant-variance option was used, the parameters (including BMD) were unchanged. BMDLs (low confidence limit of BMD, BMR = 10% RD) were 147 mg/kg-day (with minimum standard deviation) and 96.7 mg/kg-day (with maximum standard deviation); the BMDLs were within twofold, suggesting limited effect of variance in this case. Therefore, the M4 model with constant variance was used to derive the BMD and BMDL for this data set.

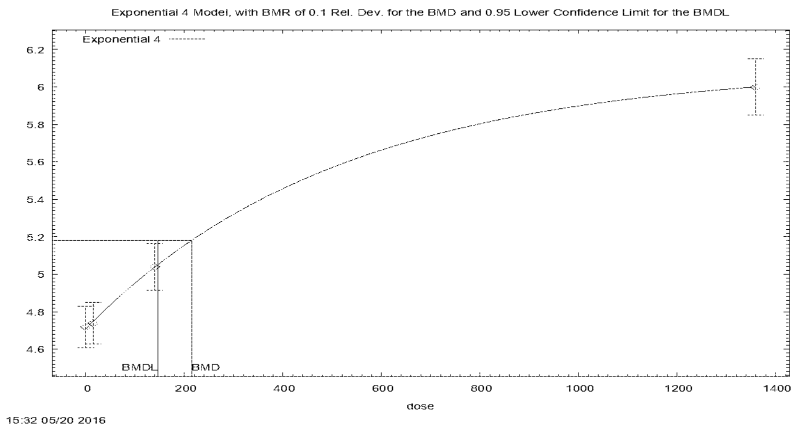
Table D-[SEQ Table * ARABIC \s 1]. Sensitivity analysis with minimum SD as variance: Summary of BMD modeling results for relative liver weight (g/100 g BW) in F2 weanling male CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA gestation and lactation {Ema, 2008, 787657}; BMR = 10% RD from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.0150	−122.66	563	512	
Exponential (M4)	**0.796**	**−128.99**	**215**	**147**	
Exponential (M5)	N/A[c]	−127.05	200	147	
Hill	N/A[c]	−127.05	207	148	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.0241	−123.60	522	468	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 1.000), selected model in bold; scaled residuals for selected model for doses 0, 14.7, 139.3, and 1,360 mg/kg-day were 0.1681, −0.1941, 0.02981, and −0.001301, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]No available degrees of freedom to calculate a goodness-of-fit value.
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e]For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from {Ema, 2008, 787657@@author-year}

ED_005297A_00166423-00129



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F2 weanling male CRL Sprague-Dawley rats exposed to HBCD during gestation and lactation on GD 0–PND 26, dose TWA gestation and lactation {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 214.961

BMDL at the 95% confidence level = 146.85

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−2.82651	−2.8274
rho	N/A	0
a	4.71128	4.484
b	0.00192508	0.00133871
c	1.29509	1.405
d	N/A	1

1 **Table of Data and Estimated Values of Interest**

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	4.72	4.711	0.25	0.2434	0.1681
14.7	22	4.74	4.75	0.25	0.2434	−0.1941
139.3	18	5.04	5.038	0.25	0.2434	0.02981
1,360	13	6	6	0.25	0.2434	−0.001301

2
3 **Likelihoods of Interest**

Model	Log (likelihood)	Number of parameters	AIC
A1	68.52739	5	−127.0548
A2	68.53022	8	−121.0604
A3	68.52739	5	−127.0548
R	10.89708	2	−17.79415
4	68.49396	4	−128.9879

4
5 **Tests of Interest**

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	115.3	6	<0.0001
Test 2	0.00567	3	0.9999
Test 3	0.00567	3	0.9999
Test 6a	0.06685	1	0.796

6

ED_005297A_00166423-00131

Table D-[SEQ Table * ARABIC \s 1]. Sensitivity analysis with maximum SD as variance: Summary of BMD modeling results for relative liver weight (g/10 0g BW) in F2 weanling male CRL Sprague-Dawley rats exposed to HBCD by gestation and lactation on GD 0–PND 26, dose TWA gestation and lactation {Ema, 2008, 787657}; BMR = 10% RD from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.454	−0.67698	563	459	
Exponential (M4)	**0.913**	**−0.24352**	**215**	**96.7**	
Exponential (M5)	N/A[c]	1.7445	200	96.9	
Hill	N/A[c]	1.7445	207	90.2	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.498	−0.86210	522	414	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 1.000), selected model in bold; scaled residuals for selected model for doses 0, 14.7, 139.3, and 1,360 mg/kg-day were 0.07126, −0.08227, 0.01264, and −0.0005523, respectively.

[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

[c]No available degrees of freedom to calculate a goodness-of-fit value.

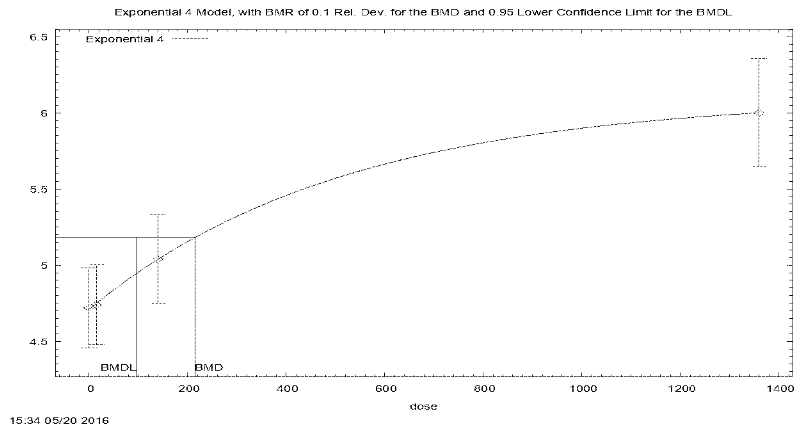
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.

[e]For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.

[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from {Ema, 2008, 787657@@author-year}

ED_005297A_00166423-00132



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F2 weanling male CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA gestation and lactation {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 214.962

BMDL at the 95% confidence level = 96.7112

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−1.10991	−1.11007
rho	N/A	0
a	4.71128	4.484
b	0.00192507	0.00133871
c	1.29509	1.405
d	N/A	1

ED_005297A_00166423-00133

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	4.72	4.711	0.59	0.5741	0.07126
14.7	22	4.74	4.75	0.59	0.5741	−0.08227
139.3	18	5.04	5.038	0.59	0.5741	0.01264
1,360	13	6	6	0.59	0.5741	−0.0005523

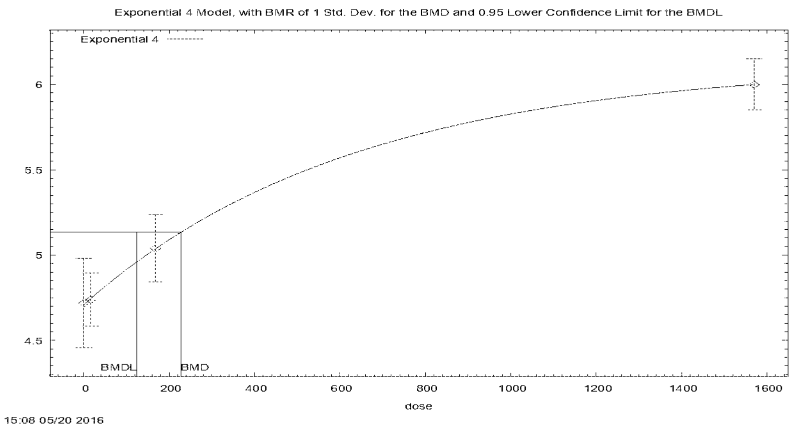
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	4.127765	5	1.744471
A2	4.130599	8	7.738801
A3	4.127765	5	1.744471
R	−14.77144	2	33.54287
4	4.121761	4	−0.2435229

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	37.8	6	<0.0001
Test 2	0.00567	3	0.9999
Test 3	0.00567	3	0.9999
Test 6a	0.01201	1	0.9127

ED_005297A_00166423-00134



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F2 weanling male CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA gestation and lactation {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 1.0000 Estimated SDs from control

BMD = 227.183

BMDL at the 95% confidence level = 124.503

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−1.72556	−1.72578
rho	N/A	0
a	4.71255	4.484
b	0.00156899	0.00115941
c	1.29864	1.405
d	N/A	1

ED_005297A_00166423-00135

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	4.72	4.713	0.59	0.422	0.08283
16.5	22	4.74	4.749	0.35	0.422	−0.09464
168	18	5.04	5.039	0.4	0.422	0.01356
1,570	13	6	6	0.25	0.422	−0.0006035

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	27.21664	5	−44.43327
A2	33.77721	8	−51.55442
A3	27.21664	5	−44.43327
R	−2.570126	2	9.140253
4	27.20864	4	−46.41727

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	72.69	6	<0.0001
Test 2	13.12	3	0.004382
Test 3	13.12	3	0.004382
Test 6a	0.016	1	0.8993

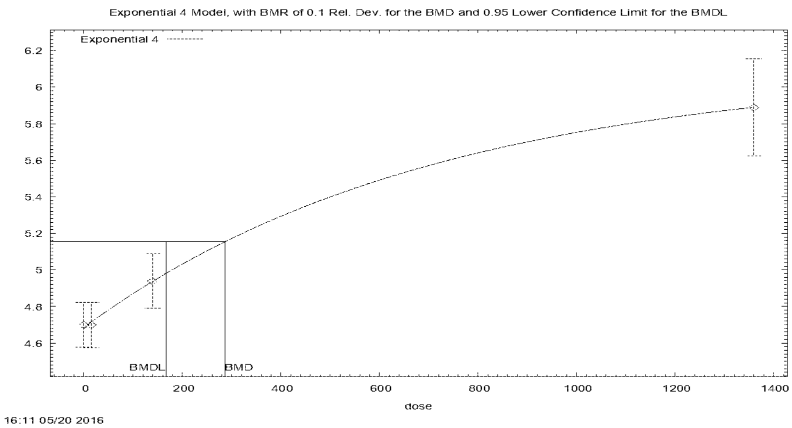
ED_005297A_00166423-00136

Table D-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for relative liver weight (g/100 g BW) in F2 weanling female CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose as TWA of gestation and lactation {Ema, 2008, 787657}; BMR = 10% RD from control mean and 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2) Exponential (M3)[b]	0.265	−92.639	589	520	400	339	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential M4 constant variance model was selected based on lowest BMDL (BMDLs differed by >3).
Exponential (M4)	**0.759**	**−93.205**	**286**	**166**	**177**	**103**	
Exponential (M5)	N/A[c]	−91.299	168	141	149	104	
Hill	N/A[c]	−91.299	153	error[d]	144	101	
Power[e] Polynomial 3°[f] Polynomial 2°[g] Linear	0.323	−93.039	549	477	367	307	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.192), selected model in bold; scaled residuals for selected model for doses 0, 14.7, 139.3, and 1,360 mg/kg-day were 0.2031, −0.2277, 0.03152, and −0.001049, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]No available degrees of freedom to calculate a goodness-of-fit value.
[d]BMD or BMDL computation failed for this model.
[e]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[f]For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[g]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from {Ema, 2008, 787657@@author-year}



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F2 weanling female CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose as TWA of gestation and lactation {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 286.259

BMDL at the 95% confidence level = 166.437

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−2.33164	−2.33288
rho	N/A	0
a	4.68619	4.465
b	0.00140932	0.00130926
c	1.30123	1.38511
d	N/A	1

This document is a draft for review purposes only and does not constitute Agency policy.

ED_005297A_00166423-00138

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	21	4.7	4.686	0.27	0.3117	0.2031
14.7	22	4.7	4.715	0.28	0.3117	−0.2277
139.3	20	4.94	4.938	0.32	0.3117	0.03152
1,360	13	5.89	5.89	0.44	0.3117	−0.001049

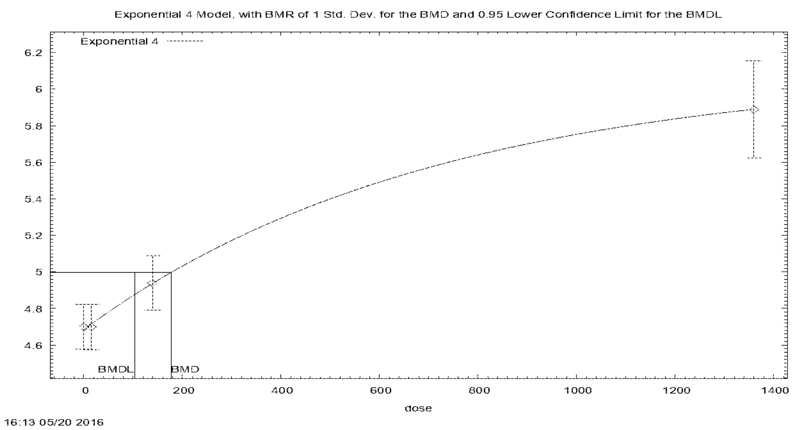
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	50.6495	5	−91.299
A2	53.0199	8	−90.03981
A3	50.6495	5	−91.299
R	9.931909	2	−15.86382
4	50.60242	4	−93.20485

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	86.18	6	<0.0001
Test 2	4.741	3	0.1918
Test 3	4.741	3	0.1918
Test 6a	0.09415	1	0.759

ED_005297A_00166423-00139



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F2 weanling female CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose as TWA of gestation and lactation {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 1.0000 Estimated SDs from control

BMD = 177.017

BMDL at the 95% confidence level = 102.961

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−2.33164	−2.33288
rho	N/A	0
a	4.68619	4.465
b	0.00140932	0.00130926
c	1.30123	1.38511
d	N/A	1

ED_005297A_00166423-00140

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	21	4.7	4.686	0.27	0.3117	0.2031
14.7	22	4.7	4.715	0.28	0.3117	−0.2277
139.3	20	4.94	4.938	0.32	0.3117	0.03152
1,360	13	5.89	5.89	0.44	0.3117	−0.001049

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	50.6495	5	−91.299
A2	53.0199	8	−90.03981
A3	50.6495	5	−91.299
R	9.931909	2	−15.86382
4	50.60242	4	−93.20485

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	86.18	6	<0.0001
Test 2	4.741	3	0.1918
Test 3	4.741	3	0.1918
Test 6a	0.09415	1	0.759

Table D-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for relative liver weight (g/100 g BW) in male CRL Sprague-Dawley rats exposed to HBCD by gavage for 13 weeks {WIL Research, 2001, 787787}; BMR = 10% RD from control mean and 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Modeled with constant variance							No model showed adequate fit. Dropping highest dose is not expected to help in this case.
Exponential (M2) Exponential (M3)[b]	3.14×10^{-4}	−67.830	328	283	269	219	
Exponential (M4)[c]	3.92×10^{-4}	−69.396	164	97.7	128	77.9	
Exponential (M5)[d]	3.92×10^{-4}	−69.396	164	97.7	128	77.9	

ED_005297A_00166423-00141

Model[a]	Goodness of fit		BMD$_{10RD}$ (mg/kg-d)	BMDL$_{10RD}$ (mg/kg-d)	BMD$_{1SD}$ (mg/kg-d)	BMDL$_{1SD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Hill	4.91×10^{-4}	−69.815	145	74.8	113	59.7	
Power[e] Polynomial 3°[f] Polynomial 2°[g] Linear	5.14×10^{-4}	−68.817	290	244	234	187	
Modeled with modeled variance							
Exponential (M2) Exponential (M3)[b]	0.00119	−68.721	337	295	320	245	
Exponential (M4)[c]	5.50×10^{-4}	−68.244	204	103	187	67.5	
Exponential (M5)[d]	5.50×10^{-4}	−68.244	204	103	187	67.5	
Hill	5.84×10^{-4}	−68.355	192	35.9	173	106	
Power[e] Polynomial 3°[f] Polynomial 2°[g] Linear	0.00161	−69.324	299	256	282	210	

[a]Constant variance (BMDS Test 2 *p*-value = 0.0644, BMDS Test 3 *p*-value = 0.0644) and nonconstant variance cases presented, no model was selected as a best-fitting model.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]The Exponential (M4) model may appear equivalent to the Exponential (M5) model; however, differences exist in digits not displayed in the table.
[d]The Exponential (M5) model may appear equivalent to the Exponential (M4) model; however, differences exist in digits not displayed in the table.
[e]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[f]For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[g]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from {WIL Research, 2001, 787787@@author-year}

ED_005297A_00166423-00142

Table D-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for relative liver weight (g/100 g BW) in female CRL Sprague-Dawley rats exposed to HBCD by gavage for 13 weeks {WIL Research, 2001, 787787}; BMR = 10% RD from control mean and 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Modeled with constant variance							
Exponential (M2) Exponential (M3)[b]	<0.0001	−39.545	310	261	332	267	No model showed adequate fit. Dropping highest dose is not expected to help in this case
Exponential (M4) Exponential (M5)[c]	2.59×10^{-4}	−44.035	101	56.0	106	61.8	
Hill	5.71×10^{-4}	−45.515	69.3	30.6	73.3	34.6	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	<0.0001	−40.679	270	220	287	226	
Modeled with modeled variance							
Exponential (M2) Exponential (M3)[b]	<0.0001	−38.793	319	269	374	282	
Exponential (M4) Exponential (M5)[c]	1.72×10^{-4}	−42.217	53.4	28.5	38.3	16.0	
Hill	0.00115	−45.763	39.2	20.7	26.0	11.6	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	<0.0001	−39.727	278	227	327	237	

[a]Constant variance (BMDS Test 2 *p*-value = 0.461, BMDS Test 3 *p*-value = 0.461) and nonconstant variance presented; no model was selected as a best-fitting model.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]For the Exponential (M5) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M4) model.
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e]For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from {WIL Research, 2001, 787787@@author-year}

ED_005297A_00166423-00143

D.2.3.3 *Reproductive*

Table D-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for primordial follicles in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}; BMR = 1% RD from control mean, 5% RD from control mean, and 10% RD from control mean

Model[a]	Goodness of fit		BMD_{1RD} (mg/kg-d)	$BMDL_{1RD}$ (mg/kg-d)	BMD_{5RD} (mg/kg-d)	$BMDL_{5RD}$ (mg/kg-d)	BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC							
Exponential (M2) Exponential (M3)[b]	0.0130	408.57	26.8	13.9	137	71.0	281	146	Exponential M4 constant variance selected as only model with adequate fit.
Exponential (M4)	**0.688**	**402.05**	**0.883**	**0.252**	**4.67**	**1.33**	**10.1**	**2.87**	
Exponential (M5)	N/A[c]	403.91	4.09	0.259	8.23	1.37	11.4	2.95	
Hill	N/A[c]	403.91	8.00	error[d]	9.28	1.10	9.99	2.50	
Power[e] Polynomial 2°[f] Linear Polynomial 3°[g]	0.0117	408.78	33.1	19.8	165	99.0	331	198	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.242), selected model in bold; scaled residuals for selected model for doses 0, 9.6, 96.3, and 940.7 mg/kg-day were −0.129, 0.1915, −0.2611, and 0.1987, respectively.

[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

[c]No available degrees of freedom to calculate a goodness-of-fit value.

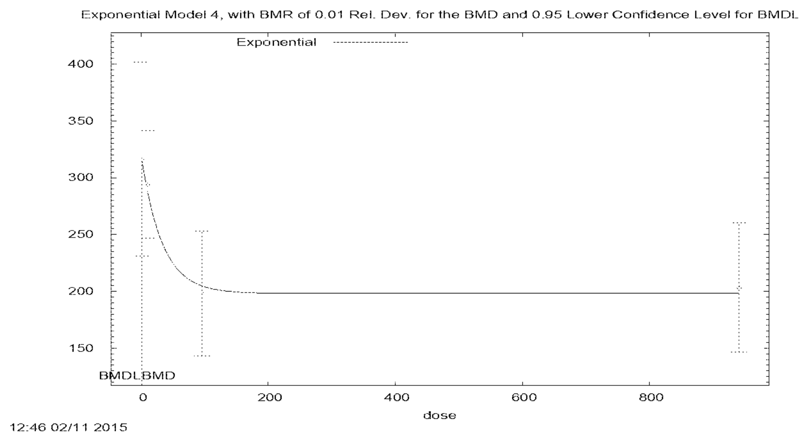
[d]BMD or BMDL computation failed for this model.

[e]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.

[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

[g]The Polynomial 3° model may appear equivalent to the Linear model; however, differences exist in digits not displayed in the table.

ED_005297A_00166423-00144



BMR = 1% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for Exponential M4, for primordial follicles in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.9; Date: 01/29/2013)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 1% RD

BMD = 0.883338

BMDL at the 95% confidence level = 0.251965

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	8.85121	8.84717
rho(S)	N/A	0
a	319.71	332.115
b	0.0301725	0.0026785
c	0.619779	0.567503
d	1	1

ED_005297A_00166423-00145

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	10	316.3	319.7	119.5	83.56	−0.129
9.6	10	294.2	289.1	66.3	83.56	0.1915
96.3	10	197.9	204.8	76.9	83.56	−0.2611
940.7	10	203.4	198.1	79.5	83.56	0.1987

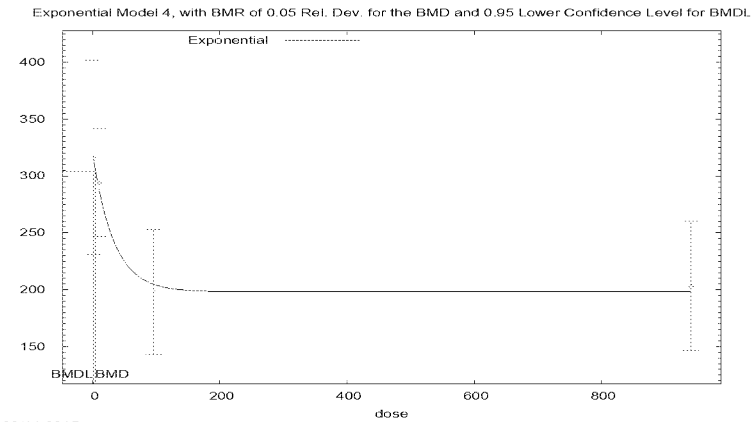
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−196.9435	5	403.8869
A2	−194.8505	8	405.701
A3	−196.9435	5	403.8869
R	−203.7104	2	411.4207
4	−197.0241	4	402.0483

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	17.72	6	0.006972
Test 2	4.186	3	0.2421
Test 3	4.186	3	0.2421
Test 6a	0.1613	1	0.6879

ED_005297A_00166423-00146



BMR = 5% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for Exponential Model 4, for primordial follicles in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.9; Date: 01/29/2013)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 5% RD

BMD = 4.67281

BMDL at the 95% confidence level = 1.32975

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	8.85121	8.84717
rho(S)	N/A	0
a	319.71	332.115
b	0.0301725	0.0026785
c	0.619779	0.567503
d	1	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	10	316.3	319.7	119.5	83.56	−0.129
9.6	10	294.2	289.1	66.3	83.56	0.1915
96.3	10	197.9	204.8	76.9	83.56	−0.2611
940.7	10	203.4	198.1	79.5	83.56	0.1987

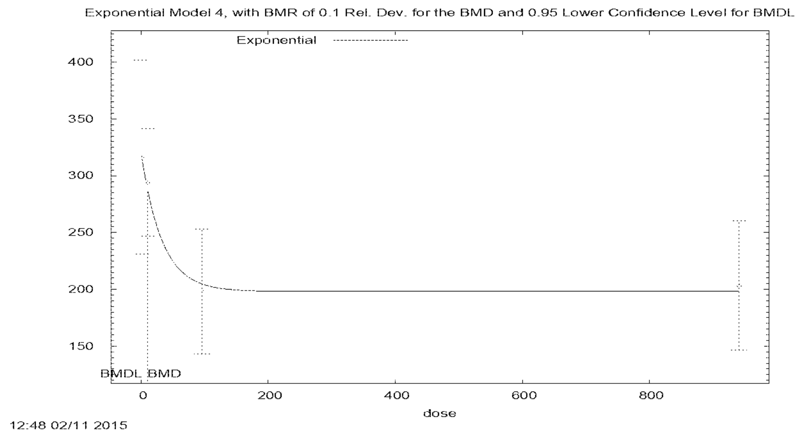
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−196.9435	5	403.8869
A2	−194.8505	8	405.701
A3	−196.9435	5	403.8869
R	−203.7104	2	411.4207
4	−197.0241	4	402.0483

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	17.72	6	0.006972
Test 2	4.186	3	0.2421
Test 3	4.186	3	0.2421
Test 6a	0.1613	1	0.6879

ED_005297A_00166423-00148



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for Exponential M4, for primordial follicles in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.9; Date: 01/29/2013)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 10.1143

BMDL at the 95% confidence level = 2.86589

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	8.85121	8.84717
rho(S)	N/A	0
a	319.71	332.115
b	0.0301725	0.0026785
c	0.619779	0.567503
d	1	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	10	316.3	319.7	119.5	83.56	−0.129
9.6	10	294.2	289.1	66.3	83.56	0.1915
96.3	10	197.9	204.8	76.9	83.56	−0.2611
940.7	10	203.4	198.1	79.5	83.56	0.1987

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−196.9435	5	403.8869
A2	−194.8505	8	405.701
A3	−196.9435	5	403.8869
R	−203.7104	2	411.4207
4	−197.0241	4	402.0483

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	17.72	6	0.006972
Test 2	4.186	3	0.2421
Test 3	4.186	3	0.2421
Test 6a	0.1613	1	0.6879

ED_005297A_00166423-00150

Table D-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for incidence of non-pregnancy in F0 and F1 CRL female rats combined exposed to HBCD in diet for 14 weeks, TWA F0 and F1 premating dose {Ema, 2008, 787657}; BMR = 5% ER and 10% ER

Model[a]	Goodness of fit		BMD_{5Pct} (mg/kg-d)	$BMDL_{5Pct}$ (mg/kg-d)	BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Gamma Weibull Multistage 3° Multistage 2° Quantal-Linear	0.0881	120.47	617	263	1,266	541	No models provided an adequate fit and a valid BMDL estimate; therefore no model was selected.
Dichotomous-Hill	N/A[b]	119.61	15.1	error[c]	35.8	13.4	
Logistic	0.0806	120.75	824	482	1,401	817	
LogLogistic	0.0897	120.43	584	230	1,232	486	
Probit	0.0815	120.72	797	449	1,392	781	
LogProbit	0.396	118.31	6.18	error[c]	159	error[c]	

[a]No model was selected as a best-fitting model.
[b]No available degrees of freedom to calculate a goodness-of-fit value.
[c]BMD or BMDL computation failed for this model.

Data from {Ema, 2008, 787657@@author-year}

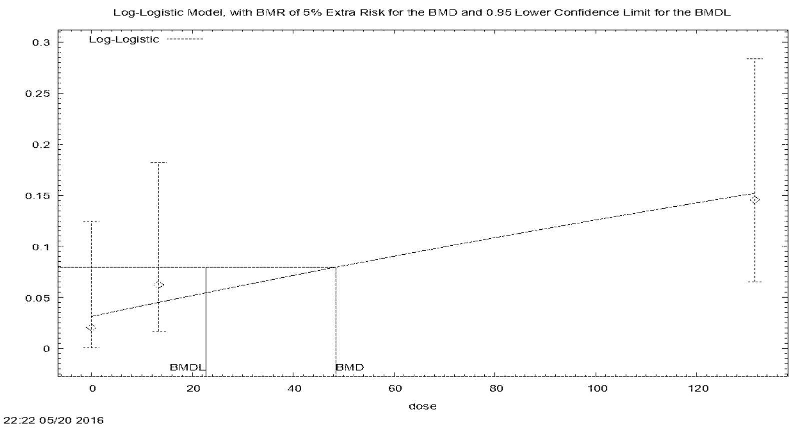
ED_005297A_00166423-00151

Table D-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for incidence of non-pregnancy in F0 and F1 CRL female rats combined exposed to HBCD in diet for 14 weeks, TWA F0 and F1 premating dose, high dose dropped {Ema, 2008, 787657}; BMR = 5% ER and 10% ER.

Model[a]	Goodness of fit		BMD_{5Pct} (mg/kg-d)	$BMDL_{5Pct}$ (mg/kg-d)	BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Gamma[b]	0.457	76.591	51.1	25.6	105	52.5	Of the models that provided an adequate fit and a valid BMDL estimate, the LogLogistic model was selected based on lowest AIC.
Logistic	0.374	76.860	77.3	53.3	121	85.5	
LogLogistic	**0.469**	**76.560**	**48.5**	**22.7**	**102**	**47.9**	
Probit	0.382	76.832	73.6	49.3	120	81.1	
LogProbit	N/A[c]	78.045	18.0	error[d]	74.8	error[d]	
Weibull[e] Quantal-Linear[f]	0.457	76.591	51.1	25.6	105	52.5	
Multistage 2°[g]	0.457	76.591	51.1	25.6	105	52.5	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 13.3, and 131.5 mg/kg-day were −0.422, 0.575, and −0.128, respectively.
[b]The Gamma model may appear equivalent to the Weibull model; however, differences exist in digits not displayed in the table. This also applies to the Multistage 2° and Quantal-Linear models.
[c]No available degrees of freedom to calculate a goodness-of-fit value.
[d]BMD or BMDL computation failed for this model.
[e]For the Weibull model, the power parameter estimate was 1. The models in this row reduced to the Quantal-Linear model.
[f]The Quantal-Linear model may appear equivalent to the Gamma model; however, differences exist in digits not displayed in the table. This also applies to the Multistage 2° model.
[g]The Multistage 2° model may appear equivalent to the Gamma model; however, differences exist in digits not displayed in the table. This also applies to the Weibull and Quantal-Linear models.

Data from {Ema, 2008, 787657@@author-year}



Dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of incidence rate by dose with fitted curve for LogLogistic model for incidence of non-pregnancy in F0 and F1 CRL female rats combined exposed to HBCD in diet for 14 weeks, TWA F0 and F1 premating dose, high dose dropped {Ema, 2008, 787657}.

Logistic Model (Version: 2.14; Date: 2/28/2013)

The form of the probability function is: P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

Slope parameter is restricted as slope >= 1

Benchmark Dose Computation

BMR = 5% ER

BMD = 48.4809

BMDL at the 95% confidence level = 22.7093

Parameter Estimates

Variable	Estimate	Default initial parameter values
background	0.0314626	0.0208333
intercept	−6.8256E+00	−6.4682E+00
slope	1	1

ED_005297A_00166423-00153

Analysis of Deviance Table

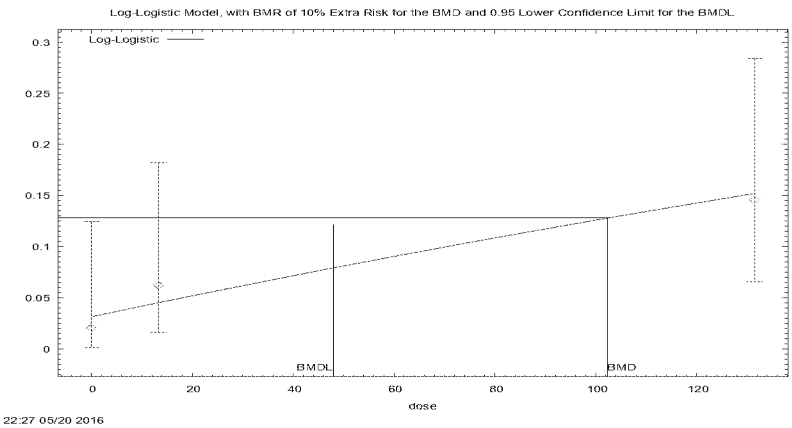
Model	Log (likelihood)	Number of parameters	Deviance	Test df	*p*-value
Full model	−36.0225	3			
Fitted model	−36.28	2	0.514904	1	0.473
Reduced model	−38.8598	1	5.6746	2	0.05858

AIC: = 76.56

Goodness-of-Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled residuals
0	0.0315	1.51	1	48	−0.422
13.3	0.0452	2.172	3	48	0.575
131.5	0.1525	7.318	7	48	−0.128

Chi^2 = 0.52, df = 1, *p*-value = 0.4687



BMR = 10% ER; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of incidence rate by dose with fitted curve for LogLogistic model for incidence of non-pregnancy in F0 and F1 CRL female rats combined exposed to HBCD in diet for 14 weeks, TWA F0 and F1 premating dose, high dose dropped {Ema, 2008, 787657}.

Logistic Model (Version: 2.14; Date: 2/28/2013)

The form of the probability function is: P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

Slope parameter is restricted as slope >= 1

ED_005297A_00166423-00154

Benchmark Dose Computation
BMR = 10% ER
BMD = 102.349
BMDL at the 95% confidence level = 47.9419

Parameter Estimates

Variable	Estimate	Default initial parameter values
background	0.0314626	0.0208333
intercept	−6.8256E+00	−6.4682E+00
slope	1	1

Analysis of Deviance Table

Model	Log (likelihood)	Number of parameters	Deviance	Test df	*p*-value
Full model	−36.0225	3			
Fitted model	−36.28	2	0.514904	1	0.473
Reduced model	−38.8598	1	5.6746	2	0.05858

AIC: = 76.56

Goodness-of-Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled residuals
0	0.0315	1.51	1	48	−0.422
13.3	0.0452	2.172	3	48	0.575
131.5	0.1525	7.318	7	48	−0.128

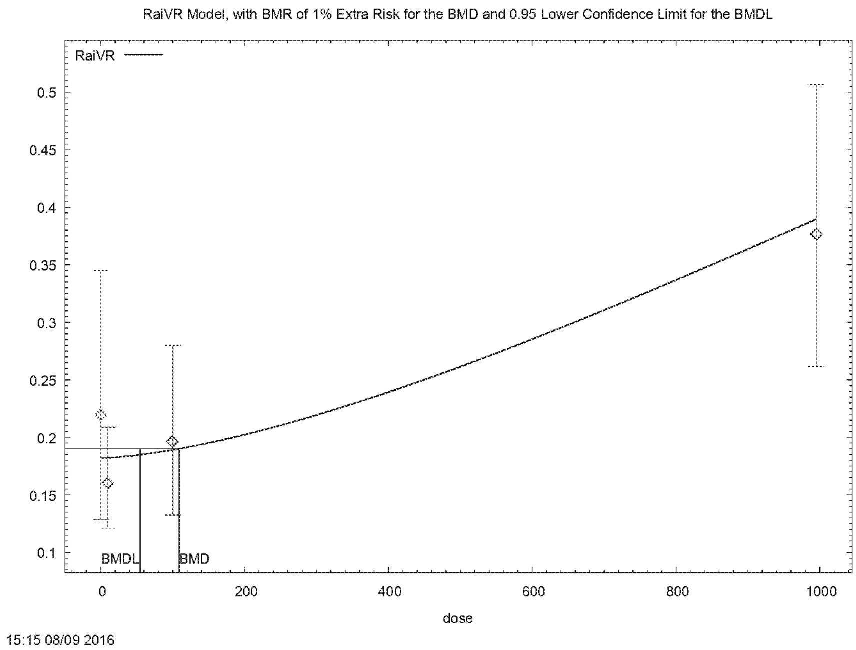
Chi^2 = 0.52, df = 1, *p*-value = 0.4687

D.2.3.4 *Developmental*

Table D-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for offspring loss from implantation through PND 4 in F2 offspring CRL Sprague-Dawley rats; gestational doses of F1 dams {Ema, 2008, 787657}; BMR = 1% ER and 5% ER

Model[a]	Goodness of Fit		BMD$_{1Pct}$ (mg/kg-d)	BMDL$_{1Pct}$ (mg/kg-d)	BMD$_{5Pct}$ (mg/kg-d)	BMDL$_{5Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Litter-specific covariate = implantation size; intra-litter correlations estimated							Of the models that provided an adequate fit, a valid BMDL estimate and BMD/BMDL <5, the NCTR/Rai and Van Ryzin model (*litter-specific covariate not used; intra-litter correlations estimated*) was selected based on lowest BMDL (BMDLs differed by >3).
Nested Logistic	0.1776	1,236.98	523.682	17.8051	708.771	92.7735	
NCTR	0.1770	1,237.29	450.409	225.409	659.055	329.826	
Rai and Van Ryzin	0.1984	1,236.26	371.593	185.81	538.091	269.046	
Litter-specific covariate = implantation size; intra-litter correlations assumed to be zero							
Nested Logistic	0.0000	1,337.62	560.759	26.8162	740.805	139.727	
NCTR	0.0000	1,335.98	553.123	460.936	739.356	616.13	
Rai and Van Ryzin	0.0000	1,337.63	138.735	86.7096	291.342	291.342	
Litter-specific covariate not used; intra-litter correlations estimated							
Nested Logistic	0.1377	1,234.32	105.863	17.0526	301.093	88.853	
NCTR[b] Rai and Van Ryzin	**0.1423**	**1,234.32**	**108.957**	**54.4786**	**315.584**	**157.792**	
Litter-specific covariate not used; intra-litter correlations assumed to be zero							
Nested Logistic	0.0000	1,336.56	132.255	25.2574	353.37	131.605	
NCTR[b] Rai and Van Ryzin	0.0000	1,336.56	136.105	68.0523	367.95	183.975	

[a]Because the individual animal data were available, the BMDS nested models were fitted, with the selected model in bold. For the selected model, the proportion of litters with scaled residuals above 2 in absolute value for doses 0, 9.7, 100, and 995 mg/kg-day were 2/23, 1/23, 1/20, and 1/21, respectively.
[b]With the litter-specific covariate not used, the NCTR and Rai and van Ryzin models yielded identical results.



BMR = 1% ER.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of incidence rate by dose, with fitted curve for the nested Rai and Van Ryzin model where the litter specific covariate was not used and the intra-litter correlations were estimated, for incidence of offspring loss from implantation through PND 4 in F2 offspring CRL Sprague-Dawley rats; gestational doses of F1 dams {Ema, 2008, 787657}.

Rai and Van Ryzin Model (Version: 2.12; Date: 04/27/2015)

The form of the probability function is:

Prob. = [1-exp(-Alpha-Beta*Dose^Rho)]*exp(-(Th1+Th2*Dose)*Rij),

where Rij is the litter specific covariate.

Restrict Power rho >= 1.

Benchmark Dose Computation

To calculate the BMD and BMDL, the litter specific covariate is fixed at the mean litter specific covariate of all the data: 14.425287

BMR = 1% ER

BMD = 108.957

BMDL at the 95% confidence level = 54.4787

ED_005297A_00166423-00157

Parameter Estimates

Variable	Estimate	(Default) Initial Parameter Values
alpha	0.201085	0.201085
beta	7.58104×10^{-6}	7.58104×10^{-6}
rho	1.53267	1.53267
phi1	0.222343	0.222343
phi2	0.0213907	0.0213907
phi3	0.0759418	0.0759418
phi4	0.277171	0.277171

Log-likelihood: −610.162 AIC: 1,234.32

Goodness-of-Fit Table

Dose	Lit.-Spec. Cov.	Est._Prob.	Litter Size	Expected	Observed	Scaled Residual
0.0000	9.0000	0.182	9	1.639	3	0.7049
0.0000	10.0000	0.182	10	1.822	4	1.0303
0.0000	11.0000	0.182	11	2.004	5	1.3037
0.0000	11.0000	0.182	11	2.004	0	-0.8718
0.0000	12.0000	0.182	12	2.186	1	-0.4778
0.0000	13.0000	0.182	13	2.368	0	-0.8885
0.0000	13.0000	0.182	13	2.368	3	0.2371
0.0000	13.0000	0.182	13	2.368	3	0.2371
0.0000	13.0000	0.182	13	2.368	0	-0.8885
0.0000	14.0000	0.182	14	2.550	1	-0.5442
0.0000	14.0000	0.182	14	2.550	3	0.1579
0.0000	15.0000	0.182	15	2.732	15	4.0466
0.0000	15.0000	0.182	15	2.732	11	2.7271
0.0000	16.0000	0.182	16	2.915	4	0.3377
0.0000	16.0000	0.182	16	2.915	2	-0.2845
0.0000	16.0000	0.182	16	2.915	2	-0.2845
0.0000	16.0000	0.182	16	2.915	1	-0.5956
0.0000	16.0000	0.182	16	2.915	2	-0.2845
0.0000	16.0000	0.182	16	2.915	2	-0.2845
0.0000	17.0000	0.182	17	3.097	3	-0.0285
0.0000	17.0000	0.182	17	3.097	0	-0.9115
0.0000	17.0000	0.182	17	3.097	6	0.8546
0.0000	18.0000	0.182	18	3.279	1	-0.6365
9.7000	2.0000	0.182	2	0.365	2	2.9630
9.7000	12.0000	0.182	12	2.188	5	1.8912
9.7000	13.0000	0.182	13	2.371	3	0.4032
9.7000	13.0000	0.182	13	2.371	0	-1.5189
9.7000	13.0000	0.182	13	2.371	4	1.0439
9.7000	14.0000	0.182	14	2.553	3	0.2736
9.7000	14.0000	0.182	14	2.553	1	-0.9508
9.7000	14.0000	0.182	14	2.553	1	-0.9508
9.7000	14.0000	0.182	14	2.553	0	-1.5630
9.7000	14.0000	0.182	14	2.553	2	-0.3386
9.7000	15.0000	0.182	15	2.735	4	0.7418
9.7000	15.0000	0.182	15	2.735	4	0.7418

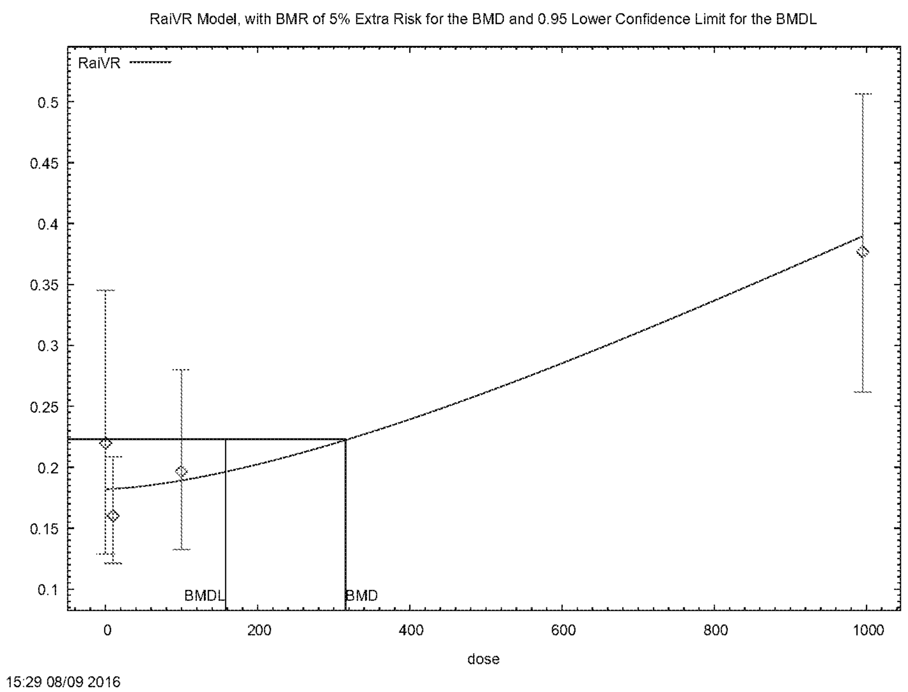
ED_005297A_00166423-00158

9.7000	15.0000	0.182	15	2.735	3	0.1552
9.7000	15.0000	0.182	15	2.735	2	-0.4314
9.7000	16.0000	0.182	16	2.918	0	-1.6437
9.7000	16.0000	0.182	16	2.918	2	-0.5170
9.7000	16.0000	0.182	16	2.918	1	-1.0803
9.7000	16.0000	0.182	16	2.918	2	-0.5170
9.7000	17.0000	0.182	17	3.100	3	-0.0543
9.7000	17.0000	0.182	17	3.100	1	-1.1386
9.7000	17.0000	0.182	17	3.100	4	0.4879
9.7000	18.0000	0.182	18	3.282	3	-0.1476
9.7000	21.0000	0.182	21	3.830	4	0.0806
100.0000	11.0000	0.189	11	2.083	3	0.5323
100.0000	11.0000	0.189	11	2.083	1	-0.6282
100.0000	12.0000	0.189	12	2.272	0	-1.2357
100.0000	13.0000	0.189	13	2.461	0	-1.2604
100.0000	14.0000	0.189	14	2.651	2	-0.3149
100.0000	14.0000	0.189	14	2.651	3	0.1691
100.0000	14.0000	0.189	14	2.651	5	1.1369
100.0000	14.0000	0.189	14	2.651	2	-0.3149
100.0000	14.0000	0.189	14	2.651	6	1.6208
100.0000	14.0000	0.189	14	2.651	1	-0.7988
100.0000	14.0000	0.189	14	2.651	2	-0.3149
100.0000	15.0000	0.189	15	2.840	1	-0.8442
100.0000	15.0000	0.189	15	2.840	2	-0.3854
100.0000	15.0000	0.189	15	2.840	0	-1.3031
100.0000	15.0000	0.189	15	2.840	3	0.0734
100.0000	16.0000	0.189	16	3.029	4	0.4235
100.0000	16.0000	0.189	16	3.029	2	-0.4491
100.0000	17.0000	0.189	17	3.219	3	-0.0910
100.0000	17.0000	0.189	17	3.219	7	1.5729
100.0000	19.0000	0.189	19	3.597	10	2.4370
995.0000	7.0000	0.393	7	2.751	7	2.0149
995.0000	10.0000	0.393	10	3.930	2	-0.6684
995.0000	11.0000	0.393	11	4.323	3	-0.4205
995.0000	12.0000	0.393	12	4.716	0	-1.3852
995.0000	12.0000	0.393	12	4.716	6	0.3772
995.0000	13.0000	0.393	13	5.109	9	1.0623
995.0000	14.0000	0.393	14	5.502	4	-0.3831
995.0000	14.0000	0.393	14	5.502	0	-1.4032
995.0000	14.0000	0.393	14	5.502	2	-0.8932
995.0000	14.0000	0.393	14	5.502	10	1.1472
995.0000	15.0000	0.393	15	5.895	8	0.5037
995.0000	15.0000	0.393	15	5.895	3	-0.6928
995.0000	15.0000	0.393	15	5.895	9	0.7430
995.0000	15.0000	0.393	15	5.895	11	1.2216
995.0000	16.0000	0.393	16	6.288	15	1.9636
995.0000	16.0000	0.393	16	6.288	4	-0.5157
995.0000	16.0000	0.393	16	6.288	2	-0.9664
995.0000	17.0000	0.393	17	6.681	6	-0.1451
995.0000	17.0000	0.393	17	6.681	1	-1.2101
995.0000	17.0000	0.393	17	6.681	5	-0.3581
995.0000	20.0000	0.393	20	7.860	6	-0.3402

Observed Chi-square = 102.1763   Bootstrap Iterations per run = 10,000

p-value = 0.1423

ED_005297A_00166423-00159



BMR = 5% ER.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of incidence rate by dose, with fitted curve for the nested Rai and Van Ryzin model where the litter specific covariate was not used and the intra-litter correlations were estimated, for incidence of offspring loss from implantation through PND 4 in F2 offspring CRL Sprague-Dawley rats; gestational doses of F1 dams {Ema, 2008, 787657}.

ED_005297A_00166423-00160

Rai and Van Ryzin Model (Version: 2.12; Date: 04/27/2015)

The form of the probability function is:

Prob. = [1-exp(-Alpha-Beta*Dose^Rho)]*exp(-(Th1+Th2*Dose)*Rij),

where Rij is the litter specific covariate.

Restrict Power rho >= 1.

Benchmark Dose Computation

To calculate the BMD and BMDL, the litter specific covariate is fixed at the mean litter specific covariate of all the data: 14.425287

BMR = 5% ER

BMD = 315.585

BMDL at the 95% confidence level = 157.792

Parameter Estimates

Variable	Estimate	(Default) Initial parameter values
alpha	0.201085	0.201085
beta	7.58104×10^{-6}	7.58104×10^{-6}
rho	1.53267	1.53267
phi1	0.222343	0.222343
phi2	0.0213907	0.0213907
phi3	0.0759418	0.0759418
phi4	0.277171	0.277171

Log-likelihood: −610.162   AIC: 1,234.32

Goodness-of-Fit Table

Dose	Lit.-Spec. Cov.	Est._Prob.	Litter Size	Expected	Observed	Scaled Residual
0.0000	9.0000	0.182	9	1.639	3	0.7049
0.0000	10.0000	0.182	10	1.822	4	1.0303
0.0000	11.0000	0.182	11	2.004	5	1.3037
0.0000	11.0000	0.182	11	2.004	0	-0.8718
0.0000	12.0000	0.182	12	2.186	1	-0.4778
0.0000	13.0000	0.182	13	2.368	0	-0.8885
0.0000	13.0000	0.182	13	2.368	3	0.2371
0.0000	13.0000	0.182	13	2.368	3	0.2371
0.0000	13.0000	0.182	13	2.368	0	-0.8885
0.0000	14.0000	0.182	14	2.550	1	-0.5442
0.0000	14.0000	0.182	14	2.550	3	0.1579
0.0000	15.0000	0.182	15	2.732	15	4.0466
0.0000	15.0000	0.182	15	2.732	11	2.7271
0.0000	16.0000	0.182	16	2.915	4	0.3377
0.0000	16.0000	0.182	16	2.915	2	-0.2845
0.0000	16.0000	0.182	16	2.915	2	-0.2845
0.0000	16.0000	0.182	16	2.915	1	-0.5956

ED_005297A_00166423-00161

0.0000	16.0000	0.182	16	2.915	2	-0.2845
0.0000	16.0000	0.182	16	2.915	2	-0.2845
0.0000	17.0000	0.182	17	3.097	3	-0.0285
0.0000	17.0000	0.182	17	3.097	0	-0.9115
0.0000	17.0000	0.182	17	3.097	6	0.8546
0.0000	18.0000	0.182	18	3.279	1	-0.6365
9.7000	2.0000	0.182	2	0.365	2	2.9630
9.7000	12.0000	0.182	12	2.188	5	1.8912
9.7000	13.0000	0.182	13	2.371	3	0.4032
9.7000	13.0000	0.182	13	2.371	0	-1.5189
9.7000	13.0000	0.182	13	2.371	4	1.0439
9.7000	14.0000	0.182	14	2.553	3	0.2736
9.7000	14.0000	0.182	14	2.553	1	-0.9508
9.7000	14.0000	0.182	14	2.553	1	-0.9508
9.7000	14.0000	0.182	14	2.553	0	-1.5630
9.7000	14.0000	0.182	14	2.553	2	-0.3386
9.7000	15.0000	0.182	15	2.735	4	0.7418
9.7000	15.0000	0.182	15	2.735	4	0.7418
9.7000	15.0000	0.182	15	2.735	3	0.1552
9.7000	15.0000	0.182	15	2.735	2	-0.4314
9.7000	16.0000	0.182	16	2.918	0	-1.6437
9.7000	16.0000	0.182	16	2.918	2	-0.5170
9.7000	16.0000	0.182	16	2.918	1	-1.0803
9.7000	16.0000	0.182	16	2.918	2	-0.5170
9.7000	17.0000	0.182	17	3.100	3	-0.0543
9.7000	17.0000	0.182	17	3.100	1	-1.1386
9.7000	17.0000	0.182	17	3.100	4	0.4879
9.7000	18.0000	0.182	18	3.282	3	-0.1476
9.7000	21.0000	0.182	21	3.830	4	0.0806
100.0000	11.0000	0.189	11	2.083	3	0.5323
100.0000	11.0000	0.189	11	2.083	1	-0.6282
100.0000	12.0000	0.189	12	2.272	0	-1.2357
100.0000	13.0000	0.189	13	2.461	0	-1.2604
100.0000	14.0000	0.189	14	2.651	2	-0.3149
100.0000	14.0000	0.189	14	2.651	3	0.1691
100.0000	14.0000	0.189	14	2.651	5	1.1369
100.0000	14.0000	0.189	14	2.651	2	-0.3149
100.0000	14.0000	0.189	14	2.651	6	1.6208
100.0000	14.0000	0.189	14	2.651	1	-0.7988
100.0000	14.0000	0.189	14	2.651	2	-0.3149
100.0000	15.0000	0.189	15	2.840	1	-0.8442
100.0000	15.0000	0.189	15	2.840	2	-0.3854
100.0000	15.0000	0.189	15	2.840	0	-1.3031
100.0000	15.0000	0.189	15	2.840	3	0.0734
100.0000	16.0000	0.189	16	3.029	4	0.4235
100.0000	16.0000	0.189	16	3.029	2	-0.4491
100.0000	17.0000	0.189	17	3.219	3	-0.0910
100.0000	17.0000	0.189	17	3.219	7	1.5729
100.0000	19.0000	0.189	19	3.597	10	2.4370
995.0000	7.0000	0.393	7	2.751	7	2.0149
995.0000	10.0000	0.393	10	3.930	2	-0.6684
995.0000	11.0000	0.393	11	4.323	3	-0.4205
995.0000	12.0000	0.393	12	4.716	0	-1.3852
995.0000	12.0000	0.393	12	4.716	6	0.3772
995.0000	13.0000	0.393	13	5.109	9	1.0623
995.0000	14.0000	0.393	14	5.502	4	-0.3831
995.0000	14.0000	0.393	14	5.502	0	-1.4032
995.0000	14.0000	0.393	14	5.502	2	-0.8932
995.0000	14.0000	0.393	14	5.502	10	1.1472
995.0000	15.0000	0.393	15	5.895	8	0.5037

ED_005297A_00166423-00162

```
995.0000   15.0000      0.393        15       5.895        3     -0.6928
995.0000   15.0000      0.393        15       5.895        9      0.7430
995.0000   15.0000      0.393        15       5.895       11      1.2216
995.0000   16.0000      0.393        16       6.288       15      1.9636
995.0000   16.0000      0.393        16       6.288        4     -0.5157
995.0000   16.0000      0.393        16       6.288        2     -0.9664
995.0000   17.0000      0.393        17       6.681        6     -0.1451
995.0000   17.0000      0.393        17       6.681        1     -1.2101
995.0000   17.0000      0.393        17       6.681        5     -0.3581
995.0000   20.0000      0.393        20       7.860        6     -0.3402
```

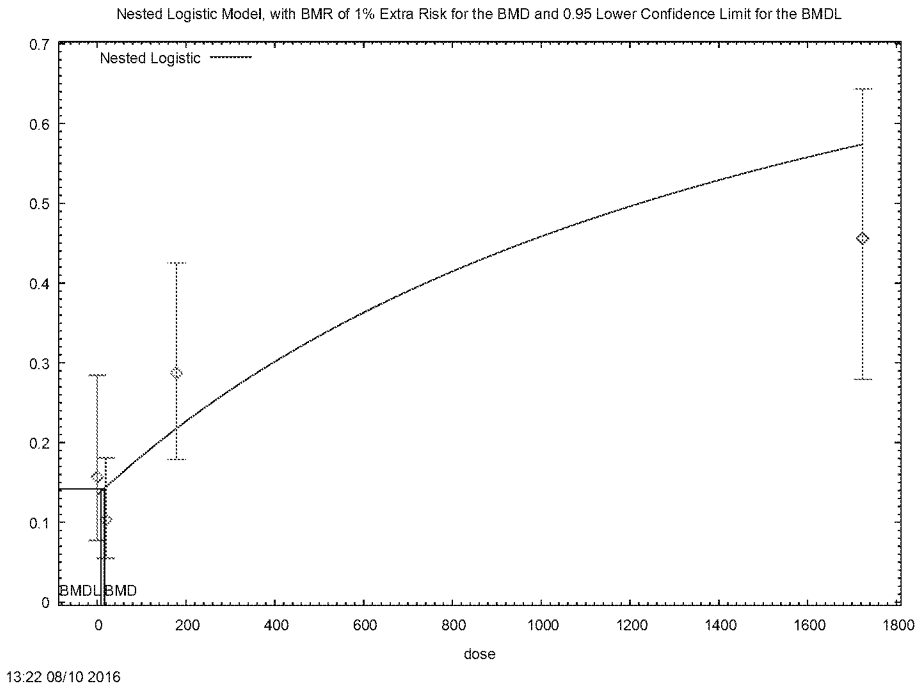
Observed Chi-square = 102.1763   Bootstrap Iterations per run = 10,000
p-value = 0.1416

Table D-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for offspring loss from PND 4 through PND 21 in F2 offspring CRL Sprague-Dawley rats; lactational doses of F1 dams {Ema, 2008, 787657}; BMR = 1% ER and 5% ER

Model[a]	Goodness of Fit		BMD_{1Pct} (mg/kg-d)	$BMDL_{1Pct}$ (mg/kg-d)	BMD_{5Pct} (mg/kg-d)	$BMDL_{5Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Litter-specific covariate = implantation size; intra-litter correlations estimated							Of the models that provided an adequate fit, a valid BMDL estimate and BMD/BMDL <5, the Nested Logistic model (*litter-specific covariate not used; intra-litter correlations estimated*) was selected based on lowest AIC (BMDLs differed by <3).
Nested Logistic	0.4417	561.04	20.4	10.1841	106.295	53.0644	
NCTR	0.4114	561.816	25.079	12.5395	127.994	63.997	
Rai and Van Ryzin	0.4056	564.38	25.8561	1.00024	131.96	5.9492	
Litter-specific covariate = implantation size; intra-litter correlations assumed to be zero							
Nested Logistic	0.0000	643.52	36.1762	22.5296	188.497	117.391	
NCTR	0.0000	650.146	33.8744	16.9372	172.883	86.4414	
Rai and Van Ryzin	0.0000	660.111	35.975	17.9875	183.603	91.8017	
Litter-specific covariate not used; intra-litter correlations estimated							
Nested Logistic	**0.3944**	**559.472**	**16.9114**	**9.03491**	**88.1172**	**47.0766**	
NCTR[b] Rai and Van Ryzin	0.4051	560.38	25.8566	12.9283	131.963	65.9814	
Litter-specific covariate not used; intra-litter correlations assumed to be zero							
Nested Logistic	0.0000	654.556	26.3666	18.3313	137.384	95.5159	
NCTR[b] Rai and Van Ryzin	0.0000	656.111	35.975	17.9875	183.603	91.8017	

[a]Because the individual animal data were available, the BMDS nested models were fitted, with the selected model in bold. For the selected model, the proportion of litters with scaled residuals above 2 in absolute value for doses 0, 19.6, 179, and 1,724 mg/kg-d were 2/22, 0/22, 2/20, and 0/20, respectively.
[b]With the litter-specific covariate not used, the NCTR and Rai and van Ryzin models yielded identical results.

ED_005297A_00166423-00163



BMR = 1% ER.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of incidence rate by dose, with fitted curve for the nested logistic model where the litter specific covariate was not used and the intra-litter correlations were estimated, for incidence of offspring loss from PND 4 through PND 21 in F2 offspring CRL Sprague-Dawley rats; lactational doses of F1 dams {Ema, 2008, 787657}.

Nested Logistic Model (Version: 2.20; Date: 04/27/2015)

The form of the probability function is:

Prob. = alpha + theta1*Rij + [1 - alpha - theta1*Rij]/
[1+exp(-beta-theta2*Rij-rho*log(Dose))],
where Rij is the litter specific covariate.

Restrict Power rho >= 1.

Benchmark Dose Computation

To calculate the BMD and BMDL, the litter specific covariate is fixed at the mean litter specific covariate of all the data: 14.654762

BMR = 1% ER

BMD = 16.9114

BMDL at the 95% confidence level = 9.03491

Parameter Estimates

Variable	Estimate	(Default) Initial Parameter Values
alpha	0.133513	0.133513
beta	−7.42311	−7.42311
rho	1	1
phi1	0.229222	0.229222
phi2	0.152985	0.152985
phi3	0.247495	0.247495
phi4	0.586386	0.586386

Log-likelihood: −273.736 AIC: 559.472

Goodness-of-Fit Table

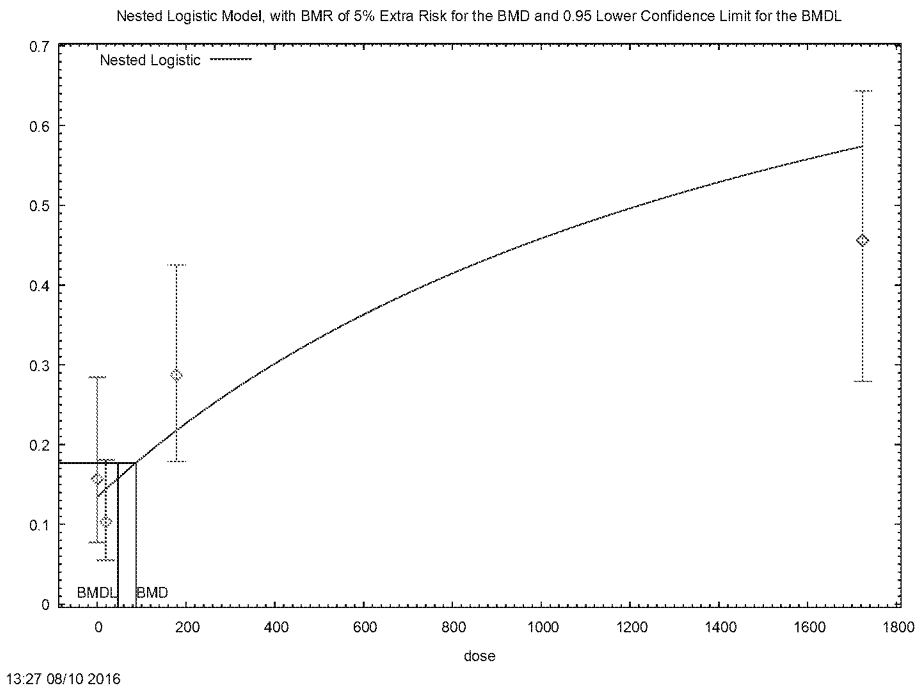
Dose	Lit.-Spec. Cov.	Est._Prob.	Litter Size	Expected	Observed	Scaled Residual
0.0000	9.0000	0.134	6	0.801	0	-0.6563
0.0000	10.0000	0.134	6	0.801	1	0.1630
0.0000	11.0000	0.134	8	1.068	0	-0.6880
0.0000	11.0000	0.134	6	0.801	0	-0.6563
0.0000	12.0000	0.134	8	1.068	1	-0.0439
0.0000	13.0000	0.134	8	1.068	6	3.1766
0.0000	13.0000	0.134	8	1.068	0	-0.6880
0.0000	13.0000	0.134	8	1.068	3	1.2443
0.0000	13.0000	0.134	8	1.068	0	-0.6880
0.0000	14.0000	0.134	8	1.068	1	-0.0439
0.0000	14.0000	0.134	8	1.068	0	-0.6880
0.0000	15.0000	0.134	4	0.534	0	-0.6043
0.0000	16.0000	0.134	8	1.068	1	-0.0439
0.0000	16.0000	0.134	8	1.068	1	-0.0439
0.0000	16.0000	0.134	8	1.068	0	-0.6880
0.0000	16.0000	0.134	8	1.068	2	0.6002
0.0000	16.0000	0.134	8	1.068	1	-0.0439
0.0000	16.0000	0.134	8	1.068	4	1.8884
0.0000	17.0000	0.134	8	1.068	0	-0.6880
0.0000	17.0000	0.134	8	1.068	0	-0.6880
0.0000	17.0000	0.134	8	1.068	5	2.5325
0.0000	18.0000	0.134	8	1.068	0	-0.6880
19.6000	12.0000	0.144	7	1.005	2	0.7747
19.6000	13.0000	0.144	8	1.148	1	-0.1039
19.6000	13.0000	0.144	8	1.148	0	-0.8046
19.6000	13.0000	0.144	8	1.148	3	1.2975
19.6000	14.0000	0.144	8	1.148	2	0.5968
19.6000	14.0000	0.144	8	1.148	0	-0.8046
19.6000	14.0000	0.144	8	1.148	0	-0.8046
19.6000	14.0000	0.144	8	1.148	0	-0.8046
19.6000	14.0000	0.144	8	1.148	0	-0.8046
19.6000	15.0000	0.144	8	1.148	1	-0.1039

ED_005297A_00166423-00165

19.6000	15.0000	0.144	8	1.148	3	1.2975
19.6000	15.0000	0.144	8	1.148	0	-0.8046
19.6000	15.0000	0.144	8	1.148	1	-0.1039
19.6000	16.0000	0.144	8	1.148	0	-0.8046
19.6000	16.0000	0.144	8	1.148	0	-0.8046
19.6000	16.0000	0.144	8	1.148	0	-0.8046
19.6000	16.0000	0.144	8	1.148	0	-0.8046
19.6000	17.0000	0.144	8	1.148	1	-0.1039
19.6000	17.0000	0.144	8	1.148	0	-0.8046
19.6000	17.0000	0.144	8	1.148	3	1.2975
19.6000	18.0000	0.144	8	1.148	1	-0.1039
19.6000	21.0000	0.144	8	1.148	0	-0.8046
179.0000	11.0000	0.217	8	1.738	4	1.1735
179.0000	11.0000	0.217	8	1.738	2	0.1361
179.0000	12.0000	0.217	8	1.738	2	0.1361
179.0000	13.0000	0.217	8	1.738	0	-0.9013
179.0000	14.0000	0.217	8	1.738	2	0.1361
179.0000	14.0000	0.217	8	1.738	5	1.6922
179.0000	14.0000	0.217	8	1.738	3	0.6548
179.0000	14.0000	0.217	8	1.738	1	-0.3826
179.0000	14.0000	0.217	8	1.738	4	1.1735
179.0000	14.0000	0.217	8	1.738	1	-0.3826
179.0000	14.0000	0.217	8	1.738	6	2.2109
179.0000	15.0000	0.217	8	1.738	0	-0.9013
179.0000	15.0000	0.217	8	1.738	0	-0.9013
179.0000	15.0000	0.217	8	1.738	1	-0.3826
179.0000	15.0000	0.217	8	1.738	6	2.2109
179.0000	16.0000	0.217	8	1.738	0	-0.9013
179.0000	16.0000	0.217	8	1.738	4	1.1735
179.0000	17.0000	0.217	8	1.738	0	-0.9013
179.0000	17.0000	0.217	8	1.738	0	-0.9013
179.0000	19.0000	0.217	8	1.738	5	1.6922
1,724.0000	10.0000	0.573	8	4.585	4	-0.1850
1,724.0000	11.0000	0.573	8	4.585	2	-0.8178
1,724.0000	12.0000	0.573	8	4.585	1	-1.1341
1,724.0000	12.0000	0.573	6	3.439	0	-1.4313
1,724.0000	13.0000	0.573	4	2.292	1	-0.7865
1,724.0000	14.0000	0.573	8	4.585	8	1.0805
1,724.0000	14.0000	0.573	8	4.585	1	-1.1341
1,724.0000	14.0000	0.573	8	4.585	0	-1.4505
1,724.0000	14.0000	0.573	4	2.292	4	1.0392
1,724.0000	15.0000	0.573	7	4.012	3	-0.3637
1,724.0000	15.0000	0.573	8	4.585	0	-1.4505
1,724.0000	15.0000	0.573	6	3.439	6	1.0662
1,724.0000	15.0000	0.573	4	2.292	4	1.0392
1,724.0000	16.0000	0.573	1	0.573	1	0.8631
1,724.0000	16.0000	0.573	8	4.585	5	0.1313
1,724.0000	16.0000	0.573	8	4.585	0	-1.4505
1,724.0000	17.0000	0.573	8	4.585	3	-0.5014
1,724.0000	17.0000	0.573	8	4.585	8	1.0805
1,724.0000	17.0000	0.573	8	4.585	3	-0.5014
1,724.0000	20.0000	0.573	8	4.585	8	1.0805

Observed Chi-square = 86.7400 Bootstrap Iterations per run = 10,000

p-value = 0.3944



BMR = 5% ER.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of incidence rate by dose, with fitted curve for the nested logistic model where the litter specific covariate was not used and the intra-litter correlations were estimated, for incidence of offspring loss from PND 4 through PND 21 in F2 offspring CRL Sprague-Dawley rats; gestational doses of F1 dams {Ema, 2008, 787657}.

Nested Logistic Model (Version: 2.20; Date: 04/27/2015)

The form of the probability function is:

Prob. = alpha + theta1*Rij + [1 - alpha - theta1*Rij]/

[1+exp(-beta-theta2*Rij-rho*log(Dose))],

where Rij is the litter specific covariate.

Restrict Power rho >= 1.

Benchmark Dose Computation

To calculate the BMD and BMDL, the litter specific covariate is fixed at the mean litter specific covariate of all the data: 14.654762

BMR = 5% ER

ED_005297A_00166423-00167

BMD = 88.1172

BMDL at the 95% confidence level = 47.0766

Parameter Estimates

Variable	Estimate	(Default) Initial Parameter Values
alpha	0.133513	0.133513
beta	−7.42311	−7.42311
rho	1	1
phi1	0.229222	0.229222
phi2	0.152985	0.152985
phi3	0.247495	0.247495
phi4	0.586386	0.586386

Log-likelihood: −273.736 AIC: 559.472

Goodness-of-Fit Table

Dose	Lit.-Spec. Cov.	Est._Prob.	Litter Size	Expected	Observed	Scaled Residual
0.0000	9.0000	0.134	6	0.801	0	-0.6563
0.0000	10.0000	0.134	6	0.801	1	0.1630
0.0000	11.0000	0.134	8	1.068	0	-0.6880
0.0000	11.0000	0.134	6	0.801	0	-0.6563
0.0000	12.0000	0.134	8	1.068	1	-0.0439
0.0000	13.0000	0.134	8	1.068	6	3.1766
0.0000	13.0000	0.134	8	1.068	0	-0.6880
0.0000	13.0000	0.134	8	1.068	3	1.2443
0.0000	13.0000	0.134	8	1.068	0	-0.6880
0.0000	14.0000	0.134	8	1.068	1	-0.0439
0.0000	14.0000	0.134	8	1.068	0	-0.6880
0.0000	15.0000	0.134	4	0.534	0	-0.6043
0.0000	16.0000	0.134	8	1.068	1	-0.0439
0.0000	16.0000	0.134	8	1.068	1	-0.0439
0.0000	16.0000	0.134	8	1.068	0	-0.6880
0.0000	16.0000	0.134	8	1.068	2	0.6002
0.0000	16.0000	0.134	8	1.068	1	-0.0439
0.0000	16.0000	0.134	8	1.068	4	1.8884
0.0000	17.0000	0.134	8	1.068	0	-0.6880
0.0000	17.0000	0.134	8	1.068	0	-0.6880
0.0000	17.0000	0.134	8	1.068	5	2.5325
0.0000	18.0000	0.134	8	1.068	0	-0.6880
19.6000	12.0000	0.144	7	1.005	2	0.7747
19.6000	13.0000	0.144	8	1.148	1	-0.1039
19.6000	13.0000	0.144	8	1.148	0	-0.8046
19.6000	13.0000	0.144	8	1.148	3	1.2975
19.6000	14.0000	0.144	8	1.148	2	0.5968
19.6000	14.0000	0.144	8	1.148	0	-0.8046
19.6000	14.0000	0.144	8	1.148	0	-0.8046
19.6000	14.0000	0.144	8	1.148	0	-0.8046
19.6000	14.0000	0.144	8	1.148	0	-0.8046
19.6000	15.0000	0.144	8	1.148	1	-0.1039

ED_005297A_00166423-00168

19.6000	15.0000	0.144	8	1.148	3	1.2975
19.6000	15.0000	0.144	8	1.148	0	-0.8046
19.6000	15.0000	0.144	8	1.148	1	-0.1039
19.6000	16.0000	0.144	8	1.148	0	-0.8046
19.6000	16.0000	0.144	8	1.148	0	-0.8046
19.6000	16.0000	0.144	8	1.148	0	-0.8046
19.6000	16.0000	0.144	8	1.148	0	-0.8046
19.6000	17.0000	0.144	8	1.148	1	-0.1039
19.6000	17.0000	0.144	8	1.148	0	-0.8046
19.6000	17.0000	0.144	8	1.148	3	1.2975
19.6000	18.0000	0.144	8	1.148	1	-0.1039
19.6000	21.0000	0.144	8	1.148	0	-0.8046

179.0000	11.0000	0.217	8	1.738	4	1.1735
179.0000	11.0000	0.217	8	1.738	2	0.1361
179.0000	12.0000	0.217	8	1.738	2	0.1361
179.0000	13.0000	0.217	8	1.738	0	-0.9013
179.0000	14.0000	0.217	8	1.738	2	0.1361
179.0000	14.0000	0.217	8	1.738	5	1.6922
179.0000	14.0000	0.217	8	1.738	3	0.6548
179.0000	14.0000	0.217	8	1.738	1	-0.3826
179.0000	14.0000	0.217	8	1.738	4	1.1735
179.0000	14.0000	0.217	8	1.738	1	-0.3826
179.0000	14.0000	0.217	8	1.738	6	2.2109
179.0000	15.0000	0.217	8	1.738	0	-0.9013
179.0000	15.0000	0.217	8	1.738	0	-0.9013
179.0000	15.0000	0.217	8	1.738	1	-0.3826
179.0000	15.0000	0.217	8	1.738	6	2.2109
179.0000	16.0000	0.217	8	1.738	0	-0.9013
179.0000	16.0000	0.217	8	1.738	4	1.1735
179.0000	17.0000	0.217	8	1.738	0	-0.9013
179.0000	17.0000	0.217	8	1.738	0	-0.9013
179.0000	19.0000	0.217	8	1.738	5	1.6922

1,724.0000	10.0000	0.573	8	4.585	4	-0.1850
1,724.0000	11.0000	0.573	8	4.585	2	-0.8178
1,724.0000	12.0000	0.573	8	4.585	1	-1.1341
1,724.0000	12.0000	0.573	6	3.439	0	-1.4313
1,724.0000	13.0000	0.573	4	2.292	1	-0.7865
1,724.0000	14.0000	0.573	8	4.585	8	1.0805
1,724.0000	14.0000	0.573	8	4.585	1	-1.1341
1,724.0000	14.0000	0.573	8	4.585	0	-1.4505
1,724.0000	14.0000	0.573	4	2.292	4	1.0392
1,724.0000	15.0000	0.573	7	4.012	3	-0.3637
1,724.0000	15.0000	0.573	8	4.585	0	-1.4505
1,724.0000	15.0000	0.573	6	3.439	6	1.0662
1,724.0000	15.0000	0.573	4	2.292	4	1.0392
1,724.0000	16.0000	0.573	1	0.573	1	0.8631
1,724.0000	16.0000	0.573	8	4.585	5	0.1313
1,724.0000	16.0000	0.573	8	4.585	0	-1.4505
1,724.0000	17.0000	0.573	8	4.585	3	-0.5014
1,724.0000	17.0000	0.573	8	4.585	8	1.0805
1,724.0000	17.0000	0.573	8	4.585	3	-0.5014
1,724.0000	20.0000	0.573	8	4.585	8	1.0805

Observed Chi-square = 86.7400 Bootstrap Iterations per run = 10,000

p-value = 0.4003

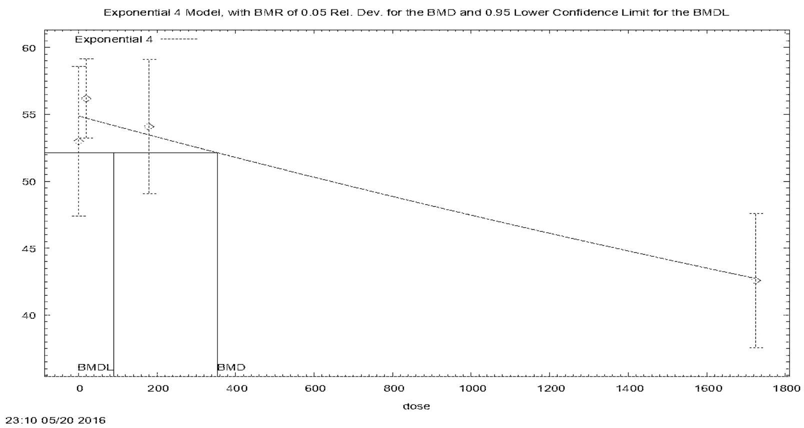
ED_005297A_00166423-00169

Table D-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for pup weight during lactation in F2 male offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose({Ema, 2008, 787657}; BMR = 5% RD from control mean, 10% RD from control mean, 0.5 SD change from control mean, and 1 SD change from control mean

Model[a]	Goodness of fit *p*-value	Goodness of fit AIC	BMD_{5RD} (mg/kg-d)	$BMDL_{5RD}$ (mg/kg-d)	BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
Exponential (M2)	0.486	420.90	354	240	727	494	Of the models that provided an adequate fit, a valid BMDL estimate and BMD/BMDL <5, the Exponential M4 constant variance model was selected based on lowest BMDL (BMDLs differed by >3).
Exponential (M3)	0.266	422.69	651	244	1016	500	
Exponential (M4)	**0.486**	**420.90**	**354**	**89.6**	**727**	**206**	
Exponential (M5)	N/A[b]	424.68	230	94.0	258	181	
Hill	N/A[b]	424.68	230	89.2	264	error[c]	
Power	0.266	422.69	676	282	1,049	565	
Polynomial 3° Polynomial 2°	0.264	422.70	817	282	1,161	564	
Linear	0.497	420.85	389	280	779	560	
Model[a]	**Goodness of fit *p*-value**	**Goodness of fit AIC**	**$BMD_{0.5SD}$ (mg/kg-d)**	**$BMDL_{0.5SD}$ (mg/kg-d)**	**BMD_{1SD} (mg/kg-d)**	**$BMDL_{1SD}$ (mg/kg-d)**	
Exponential (M2)	0.486	420.90	634	419	1,332	879	
Exponential (M3)	0.266	422.69	937	425	1,483	891	
Exponential (M4)	**0.486**	**420.90**	**634**	**172**	**1,332**	**468**	
Exponential (M5)	N/A[b]	424.68	252	176	296	189	
Hill	N/A[b]	424.68	256	176	324	error[c]	
Power	0.266	422.69	969	482	1,503	965	
Polynomial 3° **Polynomial 2°**	0.264	422.70	1,091	482	1,549	964	
Linear	0.497	420.85	684	478	1,368	956	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.0278), selected model in bold; scaled residuals for selected model for doses 0, 19.6, 179, and 1,724 mg/kg-day were −0.92, 0.71, 0.27, and −0.06, respectively.
[b]No available degrees of freedom to calculate a goodness-of-fit value.
[c]BMD or BMDL computation failed for this model.

ED_005297A_00166423-00170



BMR = 5% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for pup weight during lactation in F2 male offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 5% RD

BMD = 353.728

BMDL at the 95% confidence level = 89.5935

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	4.53195	4.51269
rho	N/A	0
a	54.8883	59.01
b	0.000145008	0.00128594
c	0	0.687535
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	53	54.89	12.6	9.64	−0.9187
19.6	22	56.2	54.73	6.7	9.64	0.714
179	18	54.1	53.48	10.1	9.64	0.272
1,724	13	42.6	42.75	8.3	9.64	−0.0551

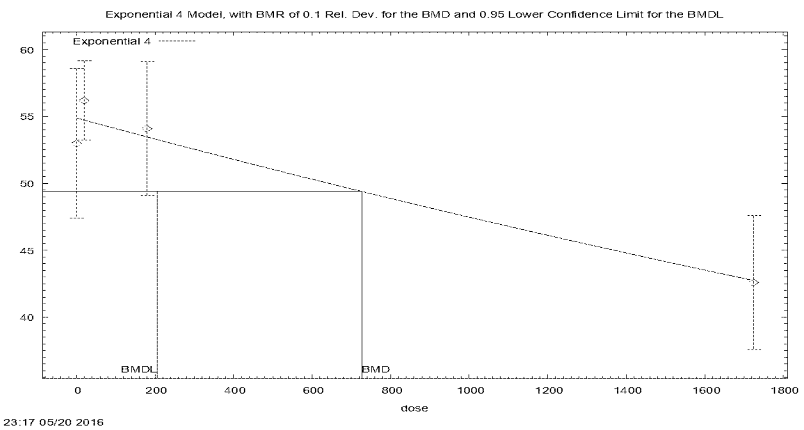
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−206.7258	5	423.4517
A2	−202.1665	8	420.333
A3	−206.7258	5	423.4517
R	−214.7267	2	433.4535
4	−207.4482	3	420.8963

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	25.12	6	0.0003244
Test 2	9.119	3	0.02775
Test 3	9.119	3	0.02775
Test 6a	1.445	2	0.4856

ED_005297A_00166423-00172



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for pup weight during lactation in F2 male offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 726.585

BMDL at the 95% confidence level = 206.377

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	4.53195	4.51269
rho	N/A	0
a	54.8883	59.01
b	0.000145008	0.00128594
c	0	0.687535
d	N/A	1

ED_005297A_00166423-00173

Table of Data and Estimated Values of Interest

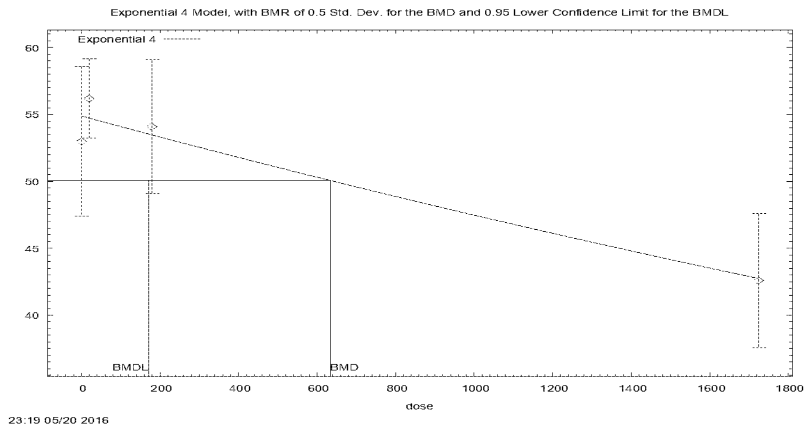
Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	53	54.89	12.6	9.64	−0.9187
19.6	22	56.2	54.73	6.7	9.64	0.714
179	18	54.1	53.48	10.1	9.64	0.272
1,724	13	42.6	42.75	8.3	9.64	−0.0551

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−206.7258	5	423.4517
A2	−202.1665	8	420.333
A3	−206.7258	5	423.4517
R	−214.7267	2	433.4535
4	−207.4482	3	420.8963

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	25.12	6	0.0003244
Test 2	9.119	3	0.02775
Test 3	9.119	3	0.02775
Test 6a	1.445	2	0.4856



BMR = 0.5 SD change from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for pup weight during lactation in F2 male offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 50% Estimated SDs from control

BMD = 633.879

BMDL at the 95% confidence level = 171.599

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	4.53195	4.51269
rho	N/A	0
a	54.8883	59.01
b	0.000145008	0.00128594
c	0	0.687535
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	53	54.89	12.6	9.64	−0.9187
19.6	22	56.2	54.73	6.7	9.64	0.714
179	18	54.1	53.48	10.1	9.64	0.272
1,724	13	42.6	42.75	8.3	9.64	−0.0551

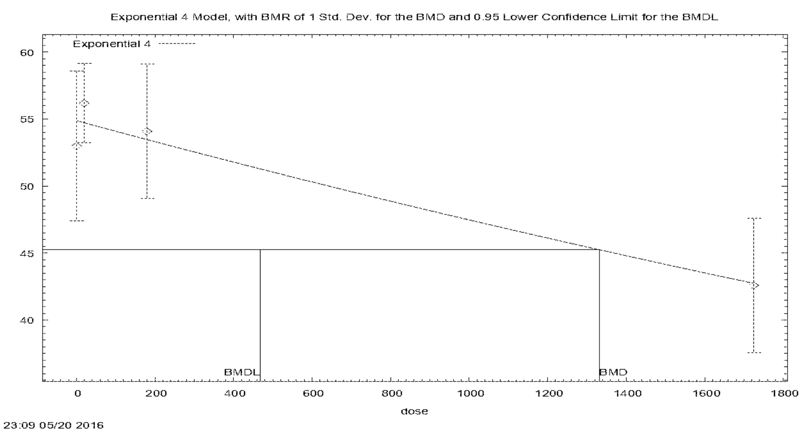
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−206.7258	5	423.4517
A2	−202.1665	8	420.333
A3	−206.7258	5	423.4517
R	−214.7267	2	433.4535
4	−207.4482	3	420.8963

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	25.12	6	0.0003244
Test 2	9.119	3	0.02775
Test 3	9.119	3	0.02775
Test 6a	1.445	2	0.4856

ED_005297A_00166423-00176



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for pup weight during lactation in F2 male offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation

BMR = 1.0000 Estimated SDs from control

BMD = 1331.98

BMDL at the 95% confidence level = 468.431

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	4.53195	4.51269
rho	N/A	0
a	54.8883	59.01
b	0.000145008	0.00128594
c	0	0.687535
d	N/A	1

ED_005297A_00166423-00177

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	53	54.89	12.6	9.64	−0.9187
19.6	22	56.2	54.73	6.7	9.64	0.714
179	18	54.1	53.48	10.1	9.64	0.272
1,724	13	42.6	42.75	8.3	9.64	−0.0551

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−206.7258	5	423.4517
A2	−202.1665	8	420.333
A3	−206.7258	5	423.4517
R	−214.7267	2	433.4535
4	−207.4482	3	420.8963

Tests of Interest

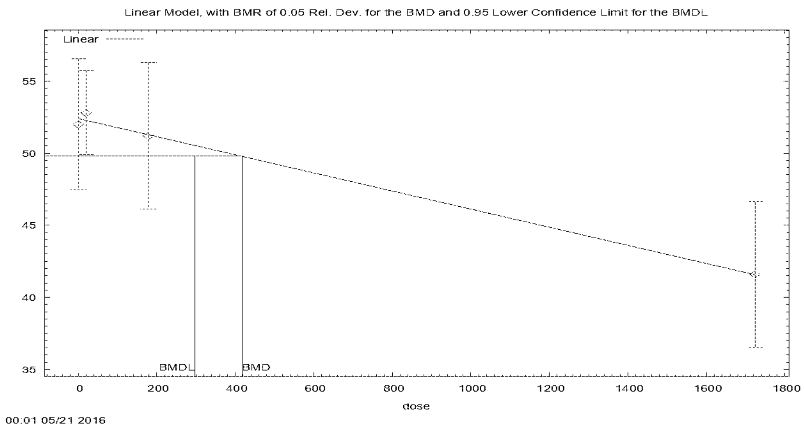
Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	25.12	6	0.0003244
Test 2	9.119	3	0.02775
Test 3	9.119	3	0.02775
Test 6a	1.445	2	0.4856

ED_005297A_00166423-00178

Table D-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for pup weight during lactation in F2 female offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose {Ema, 2008, 787657}; BMR = 5% RD from control mean, 10% RD from control mean, 0.5 SD change from control mean and 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{5RD} (mg/kg-d)	$BMDL_{5RD}$ (mg/kg-d)	BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2)	0.942	413.8640	381	257	783	528	Of the models that provided an adequate fit, a valid BMDL estimate and BMD/BMDL <5, the Linear constant variance model was selected based on lowest AIC (BMDLs differed by <3).
Exponential (M3)	0.732	415.86	411	257	815	529	
Exponential (M4)	0.729	415.86	381	257	783	528	
Exponential (M5)	N/A[b]	417.83	201	76.5	225	179	
Hill	N/A[b]	417.83	203	67.7	235	error[c]	
Power	0.729	415.86	423	297	840	594	
Polynomial 3°[c] Polynomial 2°[d] Linear	**0.942**	**413.8637**	**417**	**297**	**834**	**594**	
Model[a]	**Goodness of fit**		**$BMD_{0.5SD}$ (mg/kg-d)**	**$BMDL_{0.5SD}$ (mg/kg-d)**	**BMD_{1SD} (mg/kg-d)**	**$BMDL_{1SD}$ (mg/kg-d)**	
	***p*-value**	**AIC**					
Exponential (M2)	0.942	413.864	657	432	1378	903	
Exponential (M3)	0.732	415.86	690	432	1397	903	
Exponential (M4)	0.729	415.86	657	432	1378	903	
Exponential (M5)	N/A[b]	417.83	219	140	256	188	
Hill	N/A[b]	417.83	226	133	291	error[c]	
Power	0.729	415.86	712	489	1,416	978	
Polynomial 3° Polynomial 2° Linear	**0.942**	**413.8637**	**706**	**489**	**1,412**	**978**	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.133), selected model in bold; scaled residuals for selected model for doses 0, 19.6, 179, and 1,724 mg/kg-day were −0.22, 0.26, −0.05, and 0, respectively.
[b]No available degrees of freedom to calculate a goodness-of-fit value.
[c]BMD or BMDL computation failed for this model.



BMR = 5% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Linear model with constant variance for pup weight during lactation in F2 female offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose {Ema, 2008, 787657}.

Polynomial Model (Version: 2.20; Date: 10/22/2014)

The form of the response function is: Y[dose] = beta_0 + beta_1*dose

A constant variance model is fit

Benchmark Dose Computation

BMR = 5% RD

BMD = 417.145

BMDL at the 95% confidence level = 296.948

Parameter Estimates

Variable	Estimate	Default initial parameter values
alpha	78.7776	83.0228
rho	N/A	0
beta_0	52.4269	52.4168
beta_1	−0.00628402	−0.00627654

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	21	52	52.4	10	8.88	−0.22
19.6	22	52.8	52.3	6.6	8.88	0.262
179	20	51.2	51.3	10.8	8.88	−0.0514
1,724	13	41.6	41.6	8.4	8.88	0.00274

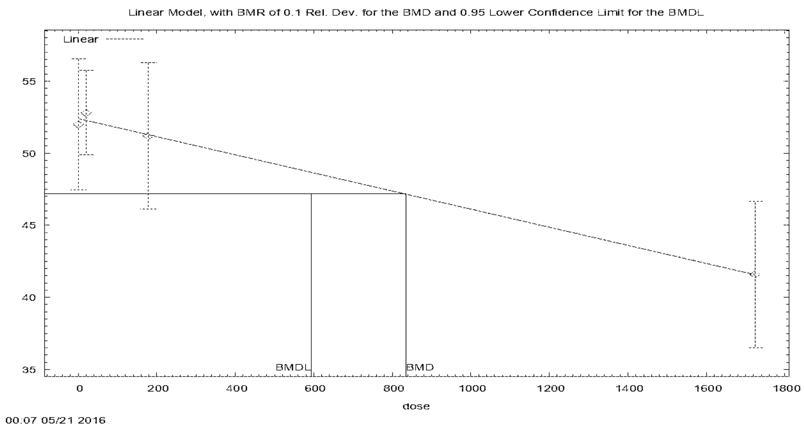
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−203.871816	5	417.743631
A2	−201.070527	8	418.141053
A3	−203.871816	5	417.743631
fitted	−203.931869	3	413.863738
R	−210.813685	2	425.627371

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	19.4863	6	0.003416
Test 2	5.60258	3	0.1326
Test 3	5.60258	3	0.1326
Test 4	0.120106	2	0.9417

ED_005297A_00166423-00181



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Linear model with constant variance for pup weight during lactation in F2 female offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose {Ema, 2008, 787657}.

Polynomial Model (Version: 2.20; Date: 10/22/2014)

The form of the response function is: Y[dose] = beta_0 + beta_1*dose

A constant variance model is fit

Benchmark Dose Computation

BMR = 10% RD

BMD = 834.289

BMDL at the 95% confidence level = 593.896

Parameter Estimates

Variable	Estimate	Default initial parameter values
alpha	78.7776	83.0228
rho	N/A	0
beta_0	52.4269	52.4168
beta_1	−0.00628402	−0.00627654

ED_005297A_00166423-00182

1 **Table of Data and Estimated Values of Interest**

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	21	52	52.4	10	8.88	−0.22
19.6	22	52.8	52.3	6.6	8.88	0.262
179	20	51.2	51.3	10.8	8.88	−0.0514
1,724	13	41.6	41.6	8.4	8.88	0.00274

2

3 **Likelihoods of Interest**

Model	Log (likelihood)	Number of parameters	AIC
A1	−203.871816	5	417.743631
A2	−201.070527	8	418.141053
A3	−203.871816	5	417.743631
fitted	−203.931869	3	413.863738
R	−210.813685	2	425.627371

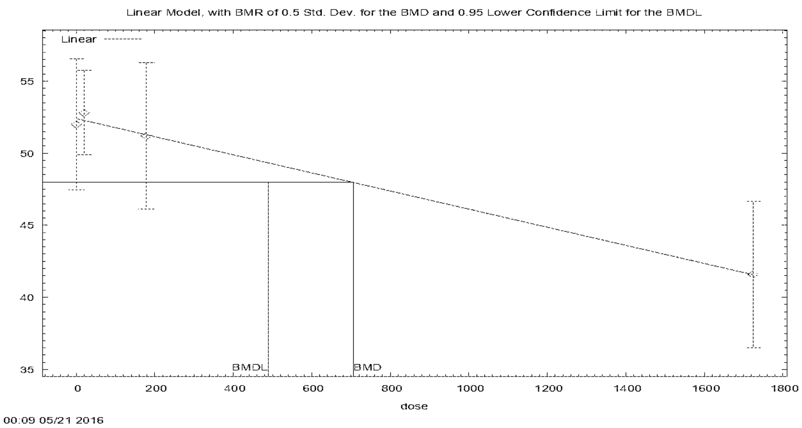
4

5 **Tests of Interest**

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	19.4863	6	0.003416
Test 2	5.60258	3	0.1326
Test 3	5.60258	3	0.1326
Test 4	0.120106	2	0.9417

6

ED_005297A_00166423-00183



BMR = 0.5 SD change from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Linear model with constant variance for pup weight during lactation in F2 female offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose {Ema, 2008, 787657}.

Polynomial Model (Version: 2.20; Date: 10/22/2014)

The form of the response function is: Y[dose] = beta_0 + beta_1*dose

A constant variance model is fit

Benchmark Dose Computation

BMR = 50% Estimated SDs from the control mean

BMD = 706.21

BMDL at the 95% confidence level = 488.985

Parameter Estimates

Variable	Estimate	Default initial parameter values
alpha	78.7776	83.0228
rho	N/A	0
beta_0	52.4269	52.4168
beta_1	−0.00628402	−0.00627654

Table of Data and Estimated Values of Interest

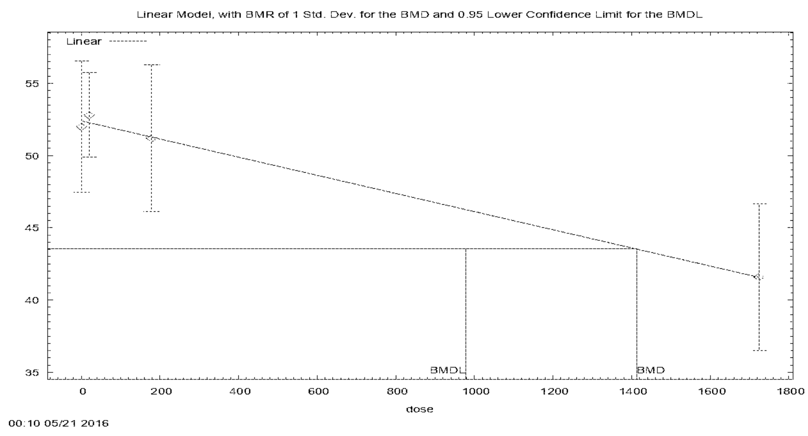
Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	21	52	52.4	10	8.88	−0.22
19.6	22	52.8	52.3	6.6	8.88	0.262
179	20	51.2	51.3	10.8	8.88	−0.0514
1,724	13	41.6	41.6	8.4	8.88	0.00274

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−203.871816	5	417.743631
A2	−201.070527	8	418.141053
A3	−203.871816	5	417.743631
fitted	−203.931869	3	413.863738
R	−210.813685	2	425.627371

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	19.4863	6	0.003416
Test 2	5.60258	3	0.1326
Test 3	5.60258	3	0.1326
Test 4	0.120106	2	0.9417



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure D-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Linear model with constant variance for pup weight during lactation in F2 female offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose {Ema, 2008, 787657}.

Polynomial Model (Version: 2.20; Date: 10/22/2014)

The form of the response function is: Y[dose] = beta_0 + beta_1*dose

A constant variance model is fit

Benchmark Dose Computation

BMR = 1 Estimated SDs from the control mean

BMD = 1412.42

BMDL at the 95% confidence level = 977.97

Parameter Estimates

Variable	Estimate	Default initial parameter values
alpha	78.7776	83.0228
rho	N/A	0
beta_0	52.4269	52.4168
beta_1	−0.00628402	−0.00627654

ED_005297A_00166423-00186

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	21	52	52.4	10	8.88	−0.22
19.6	22	52.8	52.3	6.6	8.88	0.262
179	20	51.2	51.3	10.8	8.88	−0.0514
1,724	13	41.6	41.6	8.4	8.88	0.00274

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−203.871816	5	417.743631
A2	−201.070527	8	418.141053
A3	−203.871816	5	417.743631
fitted	−203.931869	3	413.863738
R	−210.813685	2	425.627371

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	*p*-value
Test 1	19.4863	6	0.003416
Test 2	5.60258	3	0.1326
Test 3	5.60258	3	0.1326
Test 4	0.120106	2	0.9417

ED_005297A_00166423-00187

REFERENCES FOR APPENDICES

ED_005297A_00166423-00188